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PATHS OF ABSORPTION AND EXCRETION OF SODIUM TETRAIODOPHENOLPHTHALEIN*†

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DURING the course of an intravenous Graham test, a shadow appears in the large intestine, such as shown in Fig. 1. This shadow is of uniform density, of homogeneous nature. When the dye is given orally, two different types of shadows may appear in the large intestine, one similar to the above, a uniform-homogeneous shadow, the other, a shadow of increased granular density. (Fig. 2.) This latter does not appear when the dye is given intravenously. It represents unabsorbed particles, or clumps of dye which did not dissolve.

The question arises as to what is the source of this uniform, homogeneous shadow. Is it dye which has come down the common duct and passed to the large intestine? Is it dye which has been excreted by the large intestine? Is it dye which is being absorbed by the large intestine? When the dye is given orally, can any have passed through the small intestine in this uniform state without being absorbed?

Another and more important question arises as to the clinical significance of the dye in the large intestine. Were it to throw any additional light on the diagnosis of gall bladder disease, it would be very useful indeed. That the results of the oral administration of dye are not satisfactory is shown by Robins and Goldberg,[1] who in examining 1,000 cases by the oral method of administration, found the following types of gall bladder shadow: 502 cases showed good gall bladder outline 49.51 per cent, 343 cases showed

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poor gall bladder outline 33.82 per cent, 169 cases showed no gall bladder outline 16.67 per cent. In 50.49 per cent of the cases, therefore, the gall bladder showed abnormal results. The question arises as to whether the gall bladder is pathologic, or the dye not absorbed.

Case[2] makes mention of 100 cases of dye given orally. Of these, 28 had no shadows, 13 were unsatisfactory, 11 capsules failed to dissolve. Thus in 52 cases no opinion could be expressed.

From the experimental data on the absorption and excretion of the dye included in this paper, we have found that when the dye is given intrave-

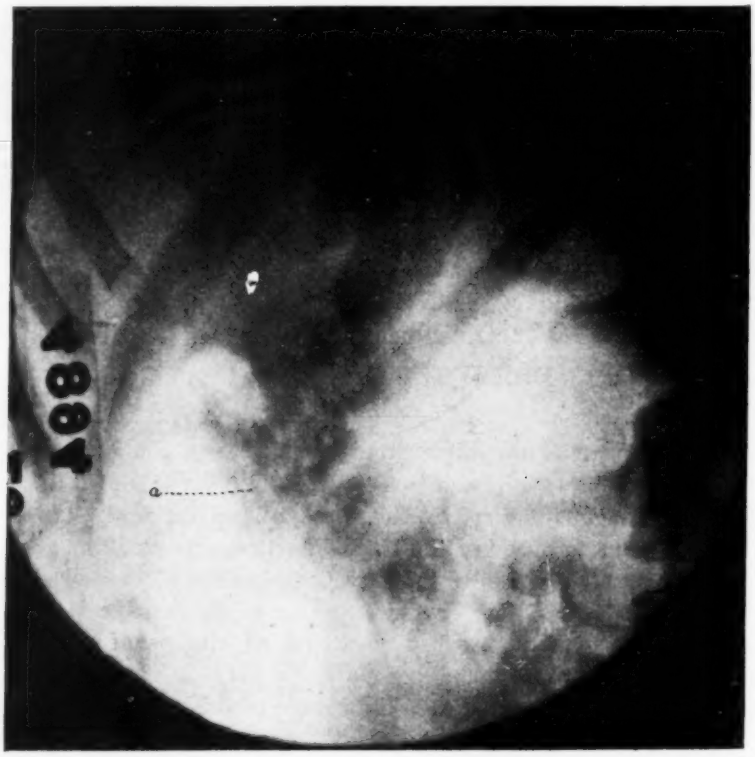


Fig. 1.—Twelve hours after the intravenous administration of the dye. An homogeneous shadow (*a*) is seen in the large intestine.

nously, about 50 to 75 per cent of the shadow in the large intestine is due to dye being eliminated from the common duct by the way of the small intestine. As we shall see later about 25 to 50 per cent of the shadow is due to dye being excreted by the large intestine. No matter how we give the dye by mouth, whether in capsules or in uniform solution, such as in grape juice, etc., the unabsorbed dye will be in a clump or in granular form and can be readily distinguished from that which is excreted by the liver or large intestine. When it is given in uniform solution, as in grape juice, the acid of the juice, as well as the hydrochloric acid of the stomach causes precipitation. Most of this precipitated dye is rendered soluble and absorbable by the alkaline juices of the small intestine, but apparently some of it is not.

The bile and dye excreted by the liver during fasting enter the gall bladder because the sphincter of Oddi is for the most part closed. The sphincter of Oddi, however, does allow some bile and dye to pass through. During the first twelve hours of fasting after an intravenous Graham test, the greatest part of the bile flows to the gall bladder. It requires food to furnish the necessary stimulation to open the sphincter of Oddi. Thus the shadow at

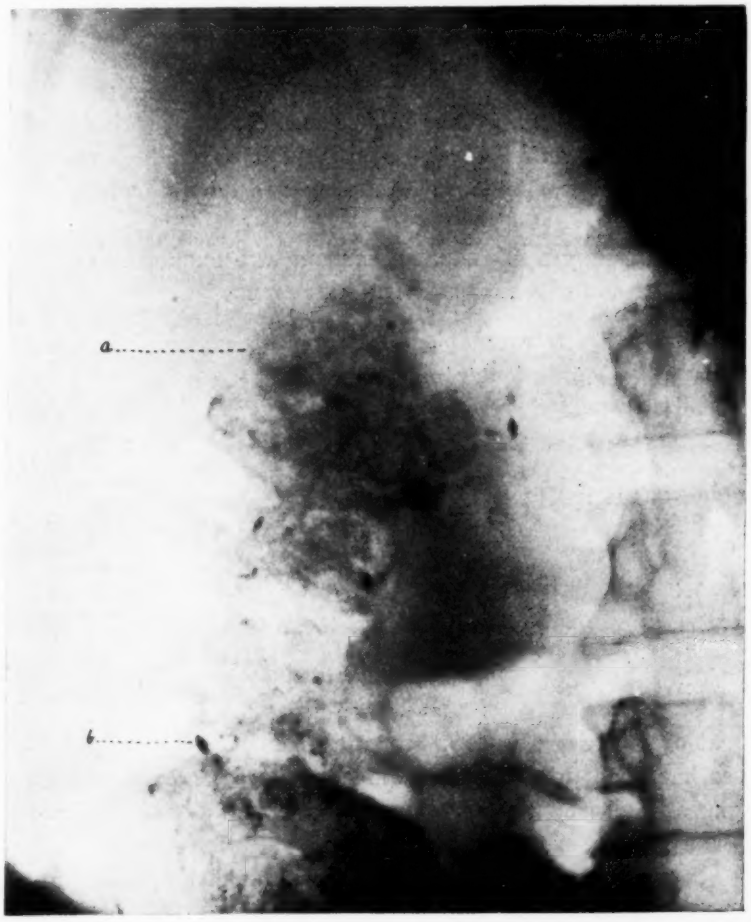


Fig. 2.—Twelve hours after the oral administration of the dye, two types of shadows seen in the large intestine *a*, homogeneous shadow, *b*, dense shadow.

this time in the large intestine represents the dye excreted by the large intestine and that found in the small portion of bile which has been excreted by the liver and has passed by the opening of the cystic duct and out from the papilla of Vater into the bowel.

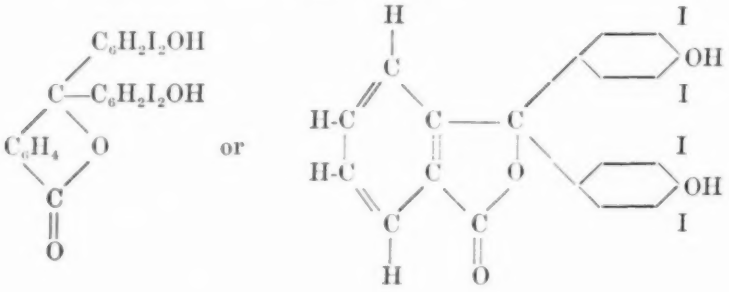
We believe that the homogeneous shadow seen in the large intestine after the intravenous administration of dye,[3] and the homogeneous shadow seen in the large intestine after the oral administration of dye, can be of assistance in the diagnosis of gall bladder disease in the following way: if

the shadow of the gall bladder and that of the large intestine are of normal density, the gall bladder and cystic duct, the common duct and the sphincter of Oddi are normally functioning; if we see the shadow in the large intestine and none in the gall bladder, there is some disturbance in the function of the gall bladder, or obstruction to the cystic or common duct (or incompetence of the sphincter of Oddi, with normal gall bladder and cystic duct); if the gall bladder shadow is weak or faint, while that of the large intestine is dense and large, we can think of either poor absorption by the gall bladder (or increased absorption), or of constriction of the cystic or common duct, or of pathologic dilatation of the hepatic and common bile ducts and incompetent sphincter.

In order to obtain more definite information concerning the possible paths of absorption and excretion of the Graham dye, animal experiments were carried out, the results of which are presented in the following.

EXPERIMENTAL DATA ON ABSORPTION AND EXCRETION OF TETRAIODOPHENOLPHTHALEIN

The sodium salt of tetraiodophenolphthalein is phenolphthalein (the basis of various laxatives) plus four atoms of iodine:



A great deal of work on the absorption and excretion of phenolphthalein derivatives has been done by Abel and Rowntree,[4] some has been done by Greenbaum[5] and also by Pratt, Doane, Harvey,[6, 7] and Behrend and Heesch;[8] but with the exception of that of Behrend and Heesch, the derivatives of phenolphthalein studied did not include tetraiodophenolphthalein.

Our experiments were conducted on dogs. We gave dye intravenously, 0.12 gm. per kilo body weight. This amount was dissolved in 5 c.c. of sterile distilled water. Our results are based upon the chemical determination of the iodine content of bile, urine, etc. The test consists in the oxidation of the organic part of our dye and the organic content of whatever contains the dye, such as blood, bile, etc. This is done by the addition of sodium hydroxide and potassium nitrate, heating, and fusing. The iodine now being in inorganic state is liberated from its salts by sulphuric acid and potassium nitrite. This is absorbed by chloroform. The color formed by absorption is compared to a known amount of potassium iodide treated with sulphuric acid and potassium nitrite and chloroform.

Rate of Disappearance of Dye From Blood Stream.—We analyzed the blood for iodine at various stages after the dye was given. As a typical illustration as to what happens to the iodine in blood, we give the following table:

1. Amount of dye given intravenously	0.96 gm. (576 mg. of I_2
2. Amount of I_2 in blood, 5 minutes later	0.04%
Amount of I_2 in blood, 15 minutes later	0.03%
Amount of I_2 in blood, 30 minutes later	0.015%
Amount of I_2 in blood, 2 hours later	Trace
Amount of I_2 in blood, 4 hours later	0.0

One can see that the blood stream gets rid of the dye in two to four hours. Occasionally dye is found in the blood stream at various hours, depending upon reabsorption of the dye after the liver has eliminated it to the gall bladder or to the small intestine.

Rate of Disappearance of the Dye From the Liver.—After the dye disappears from the blood stream one finds that the liver is filled with it. The liver eliminates it by the hepatic ducts. We studied this rate of elimination by analyzing the iodine content of the bile in dogs with common duct fistulas. In some dogs the gall bladders were removed, in others they were not. A typical case of liver elimination with the gall bladder in place is as follows:

Dog 5.—Weight, 18 pounds. Common duct fistula was made. Ninety-six-hundredths gm. of dye, containing 576 mg. of iodine, is given intravenously on first postoperative day (Table I).

TABLE I

FIRST DAY

TIME	AMOUNT OF BILE COLLECTED	PER CENT IODINE OBTAINED	MG. OF IODINE
0	Dye is given intravenously		
1 hr.	3.0 c.c.	0.0	
1½ hr.	10.0 c.c.	Trace	
2½ hr.	8.8 c.c.	0.0033	0.29
3½ hr.	2.6 c.c.	0.0200	0.52
4½ hr.	2.1 c.c.	0.1400	2.94
5½ hr.	1.4 c.c.	0.2900	4.06
7 hr.	3.0 c.c.	0.3000	9.00
8 hr.	3.1 c.c.	0.1500	4.65
9 hr.	2.0 c.c.	0.125	2.50
10 hr.	1.4 c.c.	0.109	1.53
11 hr.	2.3 c.c.	0.056	1.29
13 hr.	2.8 c.c.	0.053	1.48
14 hr.	2.4 c.c.	0.053	1.27
15 hr.	2.0 c.c.	0.0096	0.19
Urine		0	
16 hr.	3.0 c.c.	0.0033	0.10
24 hr.	3.0 c.c.	0.0030	0.10
Total			29.92

On the first day 5.19 per cent of the iodine given is recovered.

The dye is eliminated by the liver about one to two hours after it is given intravenously. It reaches its maximum concentration in the bile in about seven hours (when the gall bladder is removed this concentration is maximum in five hours, as shall be shown). In about twenty-four hours if the dog is not

fed the iodine concentration will reach what seems to be a constant from hour to hour. But so far we have recovered only 5.19 per cent of the iodine given. In this dog no iodine was found in the urine or two samples of feces on the first day.

TABLE II

SECOND DAY

TIME	SPECIMEN	PER CENT OF IODINE	MG. OF IODINE
1 hr.	Bile, 3 c.c.	0.003	0.09
1 hr.	Urine, 5 c.c.	0.0	
	Feces, 5 gm.	0.0	
	Feces, 5 gm.	0.0	
Dog ate			
2 hr.	Bile, 16.0 c.c.	0.23	36.80
4 hr.	Bile, 5.0 c.c.	0.18	9.00
6 hr.	Bile, 5.2 c.c.	0.18	9.36
8 hr.	Bile, 4.0 c.c.	0.10	4.00
24 hr.	Bile, 4.0 c.c.	0.10	4.00
Total			63.25

Iodine recovered by the second day is 16.17 per cent of total amount (Table II).

The second day shows further elimination of dye by the liver. By the giving of food the iodine concentration of the bile is greatly increased. To what is this due? Did the food cause greater liver secretion or did it cause gall bladder contraction and emptying of its contents? There is definite experimental proof that food stimulates gall bladder absorption and liver secretion.[9] Notice also that we have recovered on the second day only 16.17 per cent of the iodine given.

TABLE III

THIRD DAY

TIME	SPECIMEN	PER CENT OF IODINE	MG. OF IODINE
1 hr.	Bile, 16.6 c.c.	0.06	9.96
Urine	0.600 c.c	Faint trace	
Feces	(1) 20.0 gm.	0.07	
	(2) 20.0 gm.	0.07	
2 hr.	Bile, 10.6 c.c.	0.05	5.30
6 hr.	Bile, 15.0 c.c.	0.04	6.00
Blood	5.0 c.c.	0.0	
8 hr.	Bile, 4.8 c.c.	0.0109	5.23
Total			26.49

Only 20.82 per cent of the total iodine given is recovered in the bile.

TABLE IV

FOURTH, FIFTH, SIXTH, AND SEVENTH DAYS

TIME	SPECIMEN	PER CENT OF IODINE	MG. OF IODINE
Fourth day:			
1 hr.	Bile, 13.9 c.c.	0.002	0.28
3 hr.	Bile, 3.8 c.c.	0.0013	0.05
Fifth day:	No specimen		
Sixth day:	Bile, 18.8 c.c.	0.008	1.50
Seventh day:	Bile, 1.2 c.c.	0.000	--

On the third day (Table III) we notice that the urine, as well as the feces, contains iodine. As we shall see later about 5 to 10 per cent of the dye is eliminated in the urine. This dog has eliminated by the third day through the common duct fistula, only 20.82 per cent of the total iodine given. Another dog eliminated by the second day 36 per cent of the total iodine given. One dog eliminated by the seventh day 60 per cent of the total iodine given by vein. How does the dye get to the feces, the common duct having been led to the exterior? We must assume that the large intestine excretes it. We shall mention later the rôle of the large intestine in excretion.

On the seventh day (Table IV), for the first time, the bile no longer contained dye. Thus we see that the liver, in two to four hours after the dye is given intravenously, removes it from the blood stream, giving it off in its maximum concentration in seven hours, and giving the rest off slowly until the seventh day.

As was mentioned previously, if we remove the gall bladder and produce a common duct fistula, the iodine concentration in the bile reaches its maximal amount in five hours, and the entire elimination from the body is accomplished also in a shorter time than if the gall bladder were left in. One can see that the gall bladder tends to prolong elimination from the body and to send forward from the fifth to the seventh hour the time of maximal concentration. The liver, after an intravenous administration of dye, excretes it in increasing amounts until the fifth hour, whether the gall bladder is removed or not. At this time the gall bladder, if left in, starts to absorb the dye in greater quantities. The liver removes it from the blood stream and excretes it into the common duct. This storage of the dye by the gall bladder, with later absorption, and its re-excretion by the liver, causes the maximum concentration of the dye in the common duct to be shoved forward two hours when the gall bladder is left in place.

The following case shows the rate of excretion by the liver when the gall bladder is removed. It shows liver excretion in the earlier hours. At the fifteenth hour the animal was chloroformed and the organs were examined to see where the rest of the iodine was.

TABLE V

TIME	PER CENT OF IODINE IN THE BILE
0 hr.	Dye given intravenously
1 hr.	0.00
1½ hr.	Trace
3 hr.	0.16
4 hr.	0.33
4½ hr.	0.50
5 hr.	1.2
6½ hr.	1.0
8 hr.	1.0
8½ hr.	0.92
10½ hr.	0.81
11½ hr.	0.525
12½ hr.	0.35
13½ hr.	0.35
14½ hr.	0.30
15½ hr.	0.2

Dog 2.—Weight 18 pounds. Ninety-six-hundredths gm. of dye, or 576 mg. of iodine, were given intravenously. (Tables V and VI.)

TABLE VI

IODINE CONTENT OF VARIOUS ORGANS

Liver	Iodine present
Kidney	0
Spleen	0
Blood from heart	0
Large intestine	0
Thyroid	0.045 (0.03 normal)

The iodine was found after death in the liver and thyroid. In this dog none was found in the large intestine or urine.

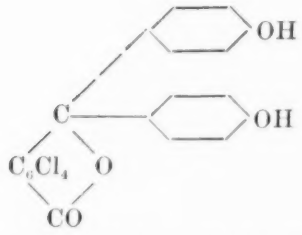
Rate of Disappearance of the Dye by the Way of the Urine.—The urine eliminates not more than 5 per cent of the dye except in cases of common duct obstruction. We analyzed urine from a woman who had taken 60 grains of dye by mouth. We obtained as follows:

April 2.		
Dye taken at 7 P.M.		
April 3.		
	Urine	
11:30 A.M.	8 oz.	5 c.c. contained trace of iodine
April 4.		
3:30 A.M.	7 oz.	5 c.c. Trace
1:40 P.M.	8½ oz.	5 c.c. Trace
5:30	8 oz.	0
7:30	14 oz.	0
9:40	15 oz.	0
April 5.		
1:30 A.M.	16 oz.	0
6:30	6 oz.	0
8:25	3½ oz.	0

Thus the urine usually eliminates all its iodine by the second day. As judged by our experience with iodine determination this trace of iodine in 700 c.c. amounted to about 5 per cent of all the iodine given.

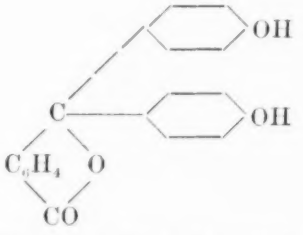
Many questions arise in studying the iodine content in a common duct fistula after dye has been given intravenously. If no more than about 50 to 75 per cent of the dye gets out by the common duct fistula and urine by the seventh day, where has the rest gone? Is the dye which has been eliminated by the common duct into the intestine reabsorbed again? If so, how does it ultimately get out of the body? Is the dye changed in its passage through the urine and liver? Does the liver conjugate it to render it insoluble?

Excretion of the Dye.—Abel and Rowntree, working on other phthaleins, have found that in the case of *phenoltetrachlorophthalein*



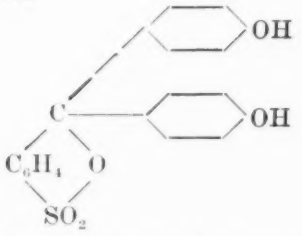
if given intravenously, it is eliminated mostly by the bile. Very little is reabsorbed by the small intestine, but it is reabsorbed by the large intestine. A little escapes with each bowel movement and in this way the dye is finally eliminated by the body in six to seven days. This dye, given by mouth, is not absorbed, given by rectum, is absorbed.

Phenolphthalein



acts in the same way except that some is absorbed by the small intestine also. When phenolphthalein is given by mouth it will be absorbed and appear in the bile and urine.

Phenolsulphonephthalein



is excreted by the bile, reabsorbed from all parts of the intestine, most at the upper end of the small intestine. Some gradually, at each reabsorption, leaves by the urine. Little, if any, leaves by the feces.

With *sodium tetraiodophenolphthalein* we have a dye which seems to combine all the features of the other dyes with known excretion. It is absorbed by mouth and by rectum, and is eliminated by the bile, and by the urine and feces.

Experimentally we found that if dye is placed in an isolated loop of the large intestine of a dog, over one-half is absorbed in twenty-four hours. (Graham test can be done by rectal administration.[10])

In six dogs, in which a loop of the large intestine was isolated and dye given intravenously two days postoperatively, we found dye in the isolated loop on the first or second day after the dye was given. In two cases more dye was found in the loop content than in the bile of the gall bladder. In one dog we found the dye in the wall of the isolated loop, but none in the content of the loop.

In five dogs we found no dye either in the wall of the isolated loop or in its content. In these cases the loops were greatly distended with fluid or gas, causing constriction of the blood supply, so no dye could be excreted.

In two dogs we removed the entire colon, and three days after the operation we gave dye intravenously and collected the fluid intestinal content by ileostomy. We thought that by removing a source of dye excretion the dye would remain in the system longer than if the colon were present. We determined this by the length of time the dye remained in the gall bladder. Three days later (seventy-two hours) the gall bladder still showed by x-ray, and contained dye in larger amounts than normally.

The large intestine thus eliminates the dye directly. In cases of obstruction of the common duct the large intestine and the kidneys are the main sources of dye elimination, and normally the 25 per cent not accounted for in bile and urine is eliminated by the large intestine, probably in such a form that it is rendered insoluble, or even if soluble, some is mechanically removed from the body in the feces.

Is the Dye Eliminated by the Liver Changed in Any Way, or Conjugated to Render It Nonabsorbable? We collected bile by a common duct fistula in dogs to which dye was given intravenously. We placed this bile-containing dye in the duodenum of a normal dog and collected bile from this dog by a common duct fistula. If the dye were changed by the liver so that it could not be reabsorbed, we would get no dye in the bile of the second dog. We collected 40 c.c. of bile containing 200 mg. of iodine from the first dog. We injected this into the duodenum of the normal dog and re-collected 66 mg. of iodine from the bile of the common duct of this dog. This shows that the dye eliminated by the liver is not rendered insoluble, probably not changed or conjugated.

Is the Dye Eliminated by the Common Duct All Reabsorbed Again by the Small Intestine? In other words, is the dye eliminated by the liver changed after it has entered the small intestine? We studied the effects of the various digestive juices on the insolubility of the dye, and found as follows (Table VII):

TABLE VII

SPECIMEN	IMMEDIATE REACTION
1. 1 c.c. of N/10 NaOH, 5 c.c. bile, 5 c.c. dye	Clear solution
2. 1 c.c. of N/10 NaOH, 5 c.c. bile, 5 c.c. pancreatin, 5 c.c. dye	Slight precipitation, insoluble in acids
3. 1 c.c. of N/10 NaOH, 5 c.c. pancreatin, 5 c.c. of dye	Clear
4. 1 c.c. of N/10 NaOH, 5 c.c. bile, 5 c.c. pancreatin, 5 c.c. dye and fat	Cloudy precipitation at junction of the fat. More than in 2.
1. 2. c.c. of N/10 HCL, 5 c.c. dye	Slight precipitation
2. 2 c.c. of N/10 HCL, bile, dye (5 c.c.)	Clear. The precipitation formed by acid is redissolved by alkali bile
3. 2 c.c. of N/10 HCL, bile, pancreatin, dye	Slight precipitation
4. 2 c.c. of N/10 HCL, bile, pancreatin, dye and fat	Loads of precipitation at fat junction

One can see that when the bile containing dye comes in contact with pancreatic juice a very slight precipitate may be caused which goes down the intestine unabsorbed. In two dogs with ileostomy we found dye which had not been absorbed by the small intestine. This may have been in this insoluble form or in an absorbable form but not absorbed. We say this because in two other dogs we obtained no dye in an ileostomy although dye was found in the upper and midde small intestine.

Action of Fat on the Solubility of the Dye.—Fat in vitro causes great precipitation at its junction with the dye. We found in dogs with a low ileostomy that a fat meal increased the amount of dye in the intestinal content. Intestinal contents were collected in the same dog before and after a fat meal. That this is not all insoluble or unabsorbable dye is shown by the fact that if this fat-dye-containing fluid is given into the duodenum of another dog, and bile is collected, this bile will contain the dye, although not much is found (3 mg. out of 54 mg. given by duodenum).

This may be one of the ways in which fat causes a diminution in the size of the dye shadow in the gall bladder.[11] Fat precipitates the dye which would ordinarily be reabsorbed to maintain the dye shadow. Also, fat causes an increase in liver secretion, thus more dye leaves the liver, and being precipitated by fat, causes a further contraction of the dye shadow in the gall bladder. The gall bladder dye shadow must be maintained by more dye entering the gall bladder. Besides this, fat may stimulate dye absorption from the gall bladder.

By some ingenious experiments Sweet[11] has shown that the dye in the gall bladder leaves the gall bladder by absorption. This dye is absorbed from the gall bladder by way of the blood stream, for we obtained the following figures in an experiment on a dog's gall bladder after dye was given intravenously the night before.

TABLE VIII

SPECIMEN	IODINE, PER CENT
1. Blood, carotid artery	0
2. Lymph from gall bladder wall	0
3. Blood from gall bladder	0.005
4. Gall bladder wall	0.01
5. Bile from gall bladder	0.53

Do the Kidneys Change the Dye? Is the dye eliminated by the kidneys changed as it passes into the urine? We believe that some of it is, because some of the iodine seems to be in the inorganic state. It will give the test by the addition of sulphuric acid, potassium nitrite, just as potassium iodide will. Some is given off in the organic state, for if we do the test by oxidizing the organic part of the urine more iodine is found than if we estimate only the inorganic type.

SUMMARY

1. The dye shadow in the large intestine during the intravenous or the oral Graham test may be of additional help in the diagnosis of gall bladder disease in the following manner:

a. If the shadow of the gall bladder and that of the large intestine is of normal density, the gall bladder, cystic duct, and common duct are free from obstruction.

b. If the gall bladder shadow is absent or faint, while that of the large intestine is dense, we can think of poor absorption by the gall bladder (or increased absorption), constriction of the cystic or common duct, or pathologic dilatation of the hepatic or bile ducts, or incompetence of the sphincter of Oddi.

2. Experimental work on the paths of absorption and excretion of sodium tetraiodophenolphthalein reveals the following facts:

a. Dye is absorbed by both the small and the large intestine after oral or rectal administration.

b. Dye given intravenously leaves the blood stream in two to four hours. An occasional trace is found later as it is reabsorbed from the intestine or gall bladder.

c. The liver starts to excrete it in one to two hours. It reaches its maximum concentration in the bile in five hours without the gall bladder, and in seven hours with a gall bladder in situ.

d. The liver will excrete the dye a little at a time for six or seven days.

e. The liver excretes about 60 to 70 per cent of the dye, probably as sodium tetraiodophenolphthalein without changing it or conjugating it.

f. Urine excretes about 5 to 10 per cent of the dye, some in inorganic state.

g. The large intestine excretes about 25 per cent of the dye.

h. Some dye is rendered insoluble as the dye-containing bile strikes pancreatic juice.

i. Fat causes greater amount of the dye to be rendered insoluble in the small intestine. Fat causes greater liver secretion of the dye, and probably greater gall bladder absorption.

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THE EFFECT OF CALCIUM PRECIPITATING SUBSTANCES ON THE CILIATED EPITHELIUM OF THE MAXILLARY SINUS*

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STUDIES on the influence of various ions on the action of ciliated cells have attracted considerable attention since the early works of R. S. Lillie (1902-1906) who noted the effects of various electrolytes on the movement of cilia of the larva of Arenicola and excised fragments of the gills of Mytilus edulis. Lillie found that potassium ions hindered ciliary movement in Arenicola and stimulated ciliary activity in the molluses. The anions, citrate, oxalate, tartrate and sulphate, were found to be toxic; ciliary movement stops in a relatively short time following the addition of these anions to the media. Parker (1905) reported complete reversal of movement of the swimming plates in Ctenophores under the influence of potassium chloride. Gray (1922) has demonstrated that calcium is necessary for the movement of cilia of Mytilus. Thus Gray has shown that tartrates and citrates because of their affinity for calcium bring about a cessation of ciliary activity.

Although various salt solutions are widely used clinically in irrigating the maxillary sinuses, comparatively little work has been carried out on the relation between the activity and subsequent histologic changes of the ciliated epithelium and the ionic composition of the irrigating fluids. In 1928, Stark reported inflammatory changes in the mucosa of the nasal fossae and paranasal sinuses of rabbits following daily injections of 200 c.c. of tap water, physiologic saline and 2 per cent sodium chloride. He quite logically suggests that the mechanical irritation set up by such energetic procedures might have been the primary factors responsible for hyperemia, leucocytic infiltration and disappearance of cilia of the mucous membrane.

The exceedingly interesting and important studies made on the relationship between the calcium ion and the ciliated tissues of lower forms of animal life convinced me of the importance of determining the effect of calcium precipitating substances, such as oxalates, citrates and alizarin, on the lining epithelium of the maxillary sinus of mammals. It was not considered unlikely that ciliary dysfunction in the sinuses might greatly influence stagnation and infection.

Alizarin, one of the effective agents used in this investigation, is a very old drug. Fühner (1923) in reviewing the group of organic dyes states that alizarin was first isolated from madder roots in 1826 by Robiquet and Colin and prepared synthetically by Gräbe and Liebermann in 1868. The main pharmacologic action of alizarin consists in its ability to precipitate calcium. In the presence of calcium salts, purplish red insoluble calcium alizarinate ($C_{14}H_6O_2[O_2Ca] + H_2O$) is formed.

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Pollack (1928) injected varying quantities of a saturated aqueous solution of sodium alizarin sulphonate by the Chambers Microdissection method (1922) into amoeba dubia. Immediately following the injection of this reagent the amoeba rounded up and purplish red calcium alizarinate crystals were seen diffusely scattered throughout the cytoplasm while pseudopodia formation ceased.

Thirty-nine healthy rabbits eight to twelve months old and twenty-eight young albino rats which varied from 60 to 120 gm. in weight were used in this investigation. Control animals were run simultaneously with the experimental rabbits. Both experimental and control rabbits were maintained on the same diet which consisted of alfalfa hay, lettuce leaves, cabbage, and carrots. All rats received Purina dog biscuit, lettuce, and a small amount of beef liver.

The solutions used in these studies were injected at room temperature by way of the anterior nares by means of a Luer syringe and blunted hypodermic needle. The amount of fluid injected into rabbits varied from 5 to 10 c.c., while the rats received 1 to 2 c.c. All experimental animals and half of the controls that received injections of distilled water were irrigated five times at forty-eight hour intervals. Twenty-four hours after the fifth injection the animals were killed. Four experimental and four control rats were killed three months after the last injection. The mucosa from the lateral and medial walls of the maxillary sinus of the rabbits was selected for histologic examination. The entire maxillary sinus mucosa was removed from the rats for this purpose. Zenker's fluid or 10 per cent formalin was used for fixation. Paraffin sections 4μ in thickness were prepared.

Hanging drop preparations of small excised fragments of ciliated epithelium from the maxillary sinuses of rats and rabbits were examined under the microscope and the normal movement of the cilia was noted. Physiologic saline was used as the normal medium. Thirty seconds after the addition of one drop of 0.3 aqueous solution of sodium alizarin sulphonate to the medium the cilia stopped moving and numerous droplets of mucus appeared on the surface of the epithelium. Normal ciliary movement returned three minutes after the fragments had been returned to normal salt solution. No secretion of mucus was noticed at this time. The cilia were still moving though somewhat more slowly two days later. The excised tissue had been kept in sterile physiologic salt solution at room temperature.

Similar *in vitro* experiments were carried out with potassium oxalate and ammonium oxalate. In 1 per cent solution of potassium oxalate ciliary action stopped in four minutes and recovered five minutes after the fragments were returned to 0.85 per cent NaCl. Ammonium oxalate caused the cilia to stop moving in thirty-five seconds. The recovery period was somewhat longer than with potassium oxalate. Normal movement was observed twenty minutes after the tissue was returned to 0.85 per cent NaCl.

Ten young rats were irrigated with 2 c.c. of 0.3 per cent aqueous solution of sodium alizarin sulphonate every two days for ten days. Six of these rats were killed twenty-four hours after the last (fifth) irrigation. The remaining four rats of this group were killed three months later. The sinus

mucosa of those rats killed one day after the last irrigation showed a loss of cilia from the epithelium and eosinophilic infiltration in the basement membrane. No sinusitis was present. All of the four rats killed three months after the last injection of alizarin had chronic sinusitis. Histologic sections showed round cell infiltration, degeneration of the mucous glands, complete absence of cilia, exfoliation of epithelium and infiltrated eosinophiles which,

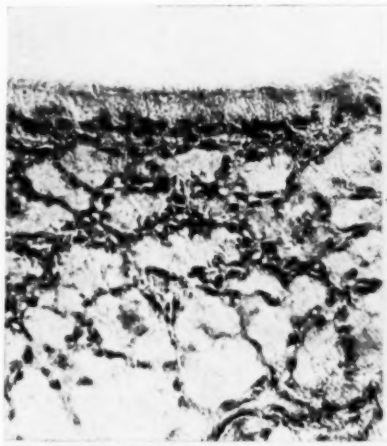

Fig. 1

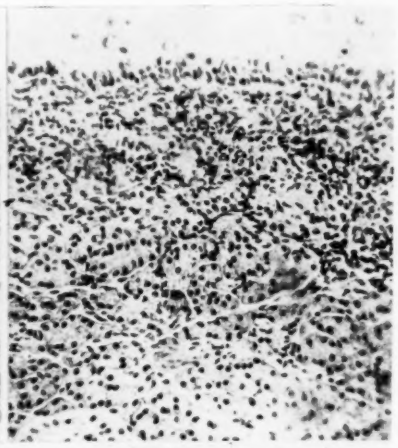

Fig. 2

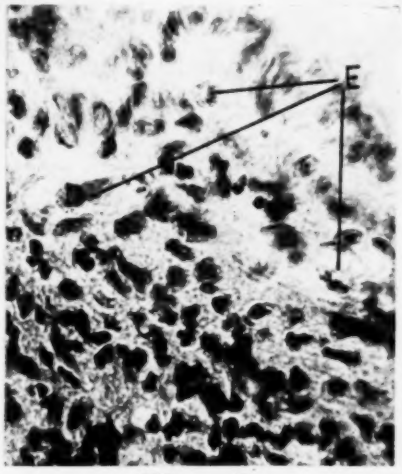

Fig. 3

Fig. 1.—Control rat 73. Normal mucous membrane from maxillary sinus twenty-four hours after the last injection of distilled water.

Fig. 2.—Experimental rat 02. Mucous membrane from maxillary sinus three months after the last injection of 0.3 per cent alizarin. Section shows desquamation of epithelium, complete absence of cilia, marked infiltration of lymphocytes and eosinophiles in basement membrane and submucosa with degeneration of mucous glands.

Fig. 3.—Same as Fig. 2, higher magnification showing eosinophiles. (*E*). Hematoxylin-eosin stain.

however, were not present in such large numbers as in the first six cases. Figs. 2 and 3 show sections taken from a rat three months after receiving the last injection of 0.3 per cent alizarin.

Eighteen rats similarly treated with 0.5 per cent solution of sodium alizarin sulphonate showed the same histologic changes in the sinus mucosa.

Fig. 1 shows the normal maxillary sinus mucosa of one of the control rats that received five irrigations with distilled water. The small amount (1 to 2 c.c.) of distilled water injected into the nasal fossae was ineffective in producing any histologic change in the mucosa of the maxillary sinus of rats. However, ciliary movement stops in about twenty seconds when the excised mucosa is placed in distilled water. Large globules of mucus appear on the surface of the epithelium. When the tissue fragments are exposed to distilled water for as long as thirty minutes to one hour and then returned to 0.85 per cent NaCl, normal ciliary action returns in approximately two minutes. The immediate osmotic effects of distilled water evidently produced no permanent tissue changes in the animal. One-half of the control rats received no treatment.

Although none of our control rats developed sinusitis, we have observed in this laboratory a large percentage of sinus infections among the old rats of our colony. Consequently only young rats were selected for this work.

Eighteen rabbits received five injections of aqueous solutions of alizarin at forty-eight hour intervals. Fig. 4 shows the infiltration of eosinophiles and nonciliated epithelium of the sinus mucosa from one of these experimental rabbits. The sinuses of this rabbit were found to be sterile. Five of the rabbits of this group had mucopurulent sinusitis.

The results obtained with alizarin suggested the use of other calcium combining anions. Sodium citrate and ammonium oxalate were selected for irrigating purposes. The former substance produced a pronounced infiltration of the eosinophilic leucocytes and partial disappearance of cilia (Fig. 5). Ammonium oxalate produced similar changes. The tissue eosinophilia, however, was not as great.

It is quite obvious that the conversion of calcium in the cell to an unionized form by the addition of alizarin, citrates or oxalates results in the destruction of ionic equilibrium with the relative increase of the other ions principal of which are the sodium and potassium ions. It is probable that the changes observed in the movement of the cilia and in the histology of the tissues are due to the sodium and potassium ions which are no longer antagonized by calcium ions. However, ciliary action is maintained for long periods in pure solutions of NaCl. In 0.5 to 1 per cent solutions of KCl the movement of the cilia is first stimulated and a large amount of mucus appears on the surface of the epithelium; the columnar cells appear to swell. In approximately two hours the cilia stopped in a contracted condition. With calcium chloride in 0.5 to 1.5 per cent solutions no apparent change in the movement of the cilia was noted.

The sinus tissue of four rabbits irrigated with 0.85 per cent solution of NaCl showed normal ciliated epithelium. The basement membrane contained large numbers of eosinophilic leucocytes. Fig. 6 shows the effect of potassium chloride on the mucous membrane of the sinus. No tissue eosinophilia was present. Calcium chloride in 0.5 to 1.5 per cent solutions produced no histologic change (Fig. 7).

Fig. 4

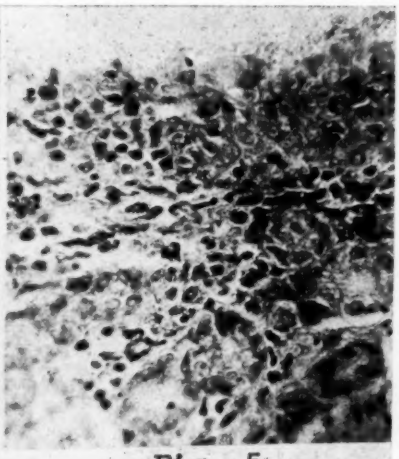

Fig. 5

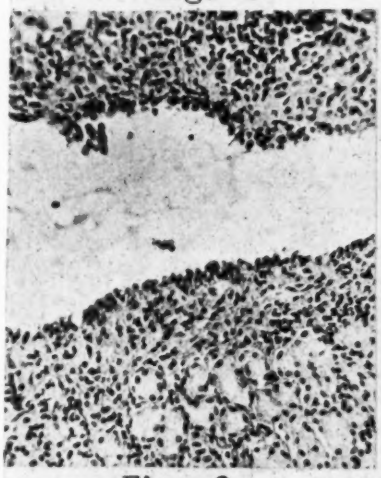

Fig. 6

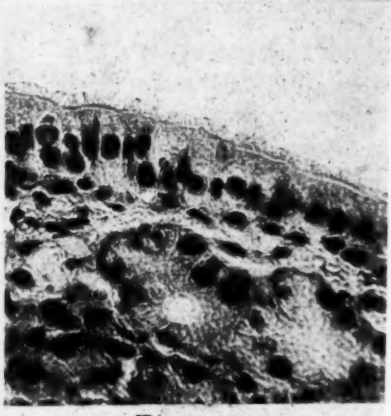

Fig. 7

Fig. 4.—Experimental rabbit 94. Twenty-four hours after last injection of 0.3 per cent alizarin. Sinus mucosa shows marked infiltration of eosinophiles in the basement membrane with emigration of eosinophiles into the lumen passing between the epithelial cells. Cilia are absent from the columnar epithelium. Giemsa stain.

Fig. 5.—Experimental rabbit 82. Twenty-four hours after last injection of sodium citrate. Section shows infiltration of eosinophiles. Most of the cilia have disappeared. Giemsa stain.

Fig. 6.—Experimental rabbit 83. Twenty-four hours after the fifth injection of 1.5 per cent potassium chloride. Basement membrane is greatly thickened. Marked infiltration of lymphocytes, no eosinophiles present. Epithelium almost entirely disappeared.

Fig. 7.—Experimental rabbit 86. Sinus mucosa following irrigations with 1.5 per cent solution of calcium chloride. Epithelium, basement membrane and submucosa entirely normal.

Although it was realized that those animals that were irrigated with distilled water do not rightfully belong in the control group they were placed there because of the histologic findings and because of the absence of ionic action (save the hydrogen ion) of the medium. It was further realized that long continued injections of distilled water might result in inflammatory changes as reported by Stark. Experiments are now being conducted on the effect of changes in P_H of the irrigating fluids on the sinus mucosa.

From the results of our experiments it appears that calcium is necessary for the normal movement of the cilia of the maxillary sinus mucosa. With the complete paralysis of the cilia by the use of calcium precipitating substances, stagnation and subsequent infection occur.

The appearance of the eosinophiles cannot be explained. From the results presented here it appears that the increase in the sodium ion in the tissue is responsible for the presence of these leucocytes. With the precipitation of calcium there occurs a relative increase in the sodium and potassium ions. Irrigation with potassium salts does not produce a tissue eosinophilia whereas isotonic sodium chloride is effective. Permeability changes, which are not to be excluded here, might possibly allow the escape of blood proteins through the vessel walls or tissue proteins through the cell walls into the surrounding fluid and thus account for the eosinophilic infiltration.

SUMMARY

1. The movement of the cilia of the maxillary sinus mucosa stops when alizarin, citrate or oxalate is added to the medium.

2. The maxillary sinuses of rabbits and rats irrigated five times at forty-eight hour intervals with sodium alizarin sulphonate showed a disappearance of cilia and infiltration of eosinophiles into the basement membrane. Three months after the last irrigation rats showed chronic sinusitis.

3. Sodium citrate and ammonium oxalate produced similar changes in the sinus mucosa of rabbits.

4. Sodium chloride produced the appearance of eosinophiles in the basement membrane of the sinus mucosa.

5. Potassium chloride irrigations resulted in marked histologic changes.

6. Calcium chloride was ineffective in producing any histologic change.

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PAROXYSMAL HEMOGLOBINURIA*

With Report of a Case

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BECAUSE it is unusual, a case of paroxysmal hemoglobinuria is reported here together with a brief review of the clinical and laboratory findings in this condition. Last year Mackenzie[1] reviewed the literature on this subject. His bibliography, containing 120 references, is comprehensive.

Definition.—Paroxysmal hemoglobinuria is a rare manifestation of late congenital and acquired syphilis. It is characterized by the presence in the blood plasma of an autohemolysin. This autohemolysin may, when the patient is exposed to cold, hemolyse enough blood cells to produce transient hemoglobinemia, hemoglobinuria, secondary anemia, evidences of altered bile metabolism, and a typical group of symptoms.

Paroxysmal hemoglobinuria should not be confused with hematuria from any cause; hematoporphrinuria from sulphonal or trional poisoning; nor with hemoglobinuria from other causes, such as: malaria, bacillary fevers, septicemia, extensive burns, transfusions of improperly matched blood, scurvy, purpura, Raynaud's disease, the so-called March hemoglobinuria, Winckel's epidemic hemoglobinuria of the newborn, and various hemolytic poisons, particularly potassium chlorate, naphthol, pyrogallol, carbon monoxide, carbon bisulphide and arseniuretted hydrogen.

Etiology and Occurrence.—The condition is rare. There are perhaps less than 300 cases on record.

It is generally accepted that true paroxysmal hemoglobinuria occurs only in syphilities. Typically, it is a manifestation of late congenital and acquired syphilis. A demonstrable autohemolysin, which will be discussed later, is present in the blood of these patients. It is interesting to note that this autohemolysin has been demonstrated repeatedly in a small percentage of late syphilities who have never shown the slightest clinical evidence of its presence.

The incidence by age, sex, and race is in proportion to that of late syphilis. Climate is important in that paroxysms are precipitated by exposure to cold. The degree of chilling necessary to produce the first paroxysm is subject to wide individual variations. Fatigue, worry and excessive indulgences seem to predispose to attacks. Subsequent attacks are sometimes brought on by less severe chilling and fatigue than the first.

Clinical Manifestations.—The symptoms are characteristic. They appear following exposure to cold of varying degree, especially if the patient is tired. They may be grouped into prodromal, paroxysmal, and postparoxysmal, as follows:

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The *prodromal* symptoms are systemic and peripheral. They appear from a few minutes to eight hours after exposure. The typical systemic symptoms are chilliness, headache, malaise, and aching pains in the back, legs or abdomen. More rarely dizziness, visual disurbances, nausea, and a desire to yawn are noted. The peripheral symptoms appear to be vasomotor. They may be indistinguishable from those of Raynaud's disease, with paresthesias and either pallor or cyanosis of the tips of the extremities. The face, lips, ears and nose may be similarly involved. There may be urticaria, erythematous blotches, or rarely, gangrenous looking purpuric patches. The prodromal stage is generally of short duration. Within a few moments the symptoms become sufficiently intensified to be termed paroxysmal.

The *paroxysm* is usually ushered in with a chill. The temperature goes up to perhaps 101° F. The systemic and vasomotor symptoms become more severe. The systolic and diastolic blood pressures may be elevated 50 to 100 mm. of mercury. After perhaps thirty minutes there will probably be an urgent desire to void. This urine, which contains free hemoglobin, will be dark red, reddish brown, or almost black. As a rule it is because of this dark urine that the patient consults a physician.

The paroxysm usually lasts from two to four hours, though occasionally for twenty-four hours or longer. The patient will ordinarily void the dark urine only in the first or first two specimens after the chill. Unusual findings are urinary frequency, dysuria, oliguria, dyspnea, or palpitation.

The postparoxysmal symptoms are apt to be merely lassitude and weakness. In many cases there is slight demonstrable jaundice due to the rapid transformation of the free hemoglobin into bile pigments. Recovery from an attack is rapid. There are no symptoms between attacks.

The terms abortive, rudimentary, frustrated, or larval attacks have been applied to those atypical paroxysms in which there is hemoglobinuria without the constitutional symptoms or in which there are the constitutional symptoms without hemoglobinuria. In this latter instance there may be a transient hemoglobinemia or bilirubinemia. It has been shown that approximately one-sixtieth of all the erythrocytes of the blood must be hemolyzed to raise the plasma hemoglobin to the renal threshold for hemoglobin excretion.

Clinical Pathology.—The more important laboratory findings are in the urine and in the blood.

The urine between attacks is normal. During a paroxysm, deep red urine due to methemoglobin, oxyhemoglobin, and partly to hematin is the characteristic finding. These substances give specific absorption bands with the spectroscope. In most attacks a few casts and erythrocytes and variable amounts of albumin are found.

Urobilin is commonly increased. Bile will be present if the concentration of bile pigments in the plasma is above the kidney threshold. Urinary chlorides, phosphates and total nitrogen have been found diminished, although urea seems to be high.

The Blood shows characteristic changes in the formed elements and in the plasma or serum.

The formed elements, i.e., erythrocytes, leucocytes, and platelets, show the following changes:

The erythrocytes decrease rapidly during a paroxysm. In severe attacks the count may decrease as much as one million cells per c.mm. in a few hours. Repeated attacks may produce severe secondary anemia. Following an attack regeneration of erythrocytes is rapid. Regeneration of hemoglobin is somewhat slower.

The leucocytes undergo rapid fluctuations. During the chilling process in artificially produced attacks there is a moderate increase in the total leucocyte count. A few moments after the chilling, after the initial appearance of free hemoglobin in the blood plasma, a leucopenia with relative lymphocytosis is found. This leucopenia, which may be rather marked, is followed by a second moderate leucocytosis with relative polymorphonucleosis within about two hours of the appearance of hemoglobinuria.

The platelets increase and decrease with the leucocytes.

The stained blood smear after an attack shows the changes of secondary anemia with regeneration such as one finds after hemorrhage.

The plasma or serum during an attack shows evidence of erythrocyte destruction which is followed by disturbed bile pigment metabolism. The autohemolysin is constantly present.

The nearly simultaneous destruction of millions of erythrocytes during a paroxysm releases free hemoglobin into the plasma, imparting to it a clear red color. The presence of free hemoglobin may be demonstrated readily with the spectroscope.

In the liver, spleen, and other organs, that part of the hemoglobin not excreted by the kidneys is converted into bile pigments. As the amount of hemoglobin in the plasma decreases the concentration of bile pigments increases. The quantitative Van den Bergh test allows a quantitative determination of this increase and reaches its peak when the free hemoglobin has entirely disappeared from the blood. This increase in bile pigments in the plasma lasts for perhaps twenty hours or longer and may be sufficient to color the skin, sclerae and urine.

Interesting observations upon the duodenal contents have shown that, coincident with the increase in bile pigments in the plasma, there is not only a marked and prompt increase in the output of bile but a simultaneous increase in urobilin and urobilinogen. This begins about one and one-half hours after chilling. The urobilin compounds are partially reabsorbed and get into the blood stream to be excreted in part by the kidneys, in part by the liver as bile. This increase in bile metabolism may be sufficient to produce a transient demonstrable enlargement of the liver and spleen.

The excessive bile pigments, bilirubin and urobilin compounds, are eliminated for the most part in the feces.

Soon after chilling a hemoclastic crisis may be observed. This term is given to a transient group of phenomena which included a decrease in clotting time, an increased viscosity of the blood, a decreased number of leucocytes and platelets, and a fall in blood pressure.

The bleeding time, clotting time (except during the hemoclastic crisis), blood calcium and phosphorous, and resistance of erythrocytes to hypotonic saline solutions are normal.

The autohemolysin is the thing which differentiates paroxysmal hemoglobinuria from all other diseases. It differs from all other known hemolytic agencies. The most acceptable theory explaining its presence is that the autohemolysin is one of the antibodies or immune bodies produced in response to the antigens liberated from the spirochetes or the syphilitic organs, or both. This is supported by the experimental production by Namba of a similar antibody in rabbits by the intraperitoneal injection of emulsions of organs of other animals.

Experimental work during the past thirty years has resulted in much information concerning the autohemolysin. Some of this is summarized below:

1. The autohemolysin is found only in syphilities.

2. It occurs in a small percentage of cases of late congenital or acquired syphilis which never show clinical evidences of paroxysmal hemoglobinuria.

3. Its presence can be demonstrated in vitro by a very simple procedure. (See diagnosis below.)

4. It is normally found in the serum or plasma and not in or attached to the cells.

5. In many respects it behaves like an amboceptor or immune body of Ehrlich.

6. It can be absorbed from the serum or plasma by erythrocytes in the cold.

7. It can be separated from the erythrocytes before being absorbed in the cold, by simple washing with normal saline at body temperature.

8. It is thermolabile, being destroyed below 0° C. in about twelve hours and sometimes between 45° and 55° C. in thirty minutes.

9. It differs from other hemolysing amboceptors or immune bodies in that the union between the autohemolysin and the erythrocytes takes place only in the cold. Room temperature, however, may be sufficiently cold to promote this union.

10. The autohemolysin-erythrocyte union will absorb complement, if present, either in the cold or at body temperature. The power to absorb complement is thought to be greater in the cold.

11. The autohemolysin-erythrocyte union, in the absence of complement, is more stabile in the cold than at body temperature. That is to say, serum separated from the erythrocytes, in a complement-free mixture, in the cold will contain fewer hemolysins than serum separated from the same mixture after warming it to 37.5° C. However if complement is present this dissociation of hemolysins from erythrocytes is less apt to occur at body temperature. In other words an hemolysin-erythrocyte-complement complex seems to be more stabile than the simple hemolysin-erythrocyte union.

12. The autohemolysin-erythrocyte union will not result in hemolysis unless complement is present, and if it is present hemolysis will occur only at body temperature. Conversely, if an autohemolysin-erythrocyte union in the cold

in the absence of complement is warmed to body temperature hemolysis will result only when complement is added.

13. The hemolysing process uses up complement.

14. Frequent attacks of hemoglobinuria may not only use up all demonstrable complement in the blood but may cause the patient's serum to become anticomplementary.

15. The hemolysin is isohemolytic as well as autohemolytic. It will hemolyse erythrocytes from human beings belonging to the same or different blood groups, thus differing from the isohemolysins normally present in the blood groups of Moss.

16. The titer of the hemolysin sometimes appears to vary from day to day.

17. In general the amount of hemolysis increases with the duration and intensity of cooling, within narrow limits. In vitro about ten minutes is thought to be the optimum time and 3° to 5° C. the optimum temperature for maximum hemolysis. Shorter periods or higher temperatures or both will probably decrease the amount but longer periods or lower temperatures will probably not increase the hemolysis.

18. The autohemolysin is not the same syphilitic immune body which absorbs complement in the Wassermann reaction. In other words, serum or plasma from which all the autohemolysin has been absorbed by erythrocytes in the cold will still give a strongly positive Wassermann.

With the above information in mind a paroxysm of hemoglobinuria may be somewhat simply explained as follows: When the patient becomes chilled there is a union between autohemolysin, erythrocytes, and complement in the peripheral capillaries. When this hemolytic system is carried back into the warmer visceral circulation, hemolysis takes place, producing hemoglobinemia. If the hemoglobinemia is sufficiently marked much of the hemoglobin will be excreted by the kidneys as such, hence hemoglobinuria.

DIAGNOSIS

The diagnosis of paroxysmal hemoglobinuria as contrasted with other hemoglobinurias listed above rests upon the following points:

1. A history of paroxysmal passage of dark red urine following exposure to cold, together with the characteristic prodromal, paroxysmal, and post-paroxysmal symptoms.

2. The demonstration of free hemoglobin in the urine during an attack.

3. Absence of hemoglobinuria between attacks.

4. A positive Wassermann or other diagnostic evidence of late syphilis.

5. The demonstration of the autohemolysin. This can be done very simply in vitro by putting about 2 c.c. of the patient's oxalated blood, kept at body temperature, into each of two small test tubes. One of these tubes is immersed in ice water at about 5° C. for about ten minutes, the other being kept in the incubator. Both tubes are then incubated at 37.5° C. for about an hour. If the hemolysin is present the plasma or serum in the tube which has been cooled will be a clear red, indicating hemolysis, whereas the plasma or serum in the tube

which has been kept at body temperature will be of a normal yellowish color. This is the Donath-Landsteiner test for the autohemolysin of paroxysmal hemoglobinuria.

One should remember that a negative test (i.e., failure of the cooled blood to hemolyze) may mean that recent attacks have used up all the patient's complement. In this case repetition of the test with the addition of a small amount of complement (1/10 to 1/4 c.c. guinea pig serum or 1/2 to 1 c.c. of human serum) to both tubes will produce hemolysis in the cooled tube. Serum which has become anticomplimentary from repeated attacks is rare. Attacks numerous enough to produce this condition could be diagnosed without the demonstration of the hemolysin.

6. The artificial production of an attack by the Rosenbach or Ehrlich tests. The Rosenbach test is simply the reproduction of the symptoms and hemoglobinemia, with or without hemoglobinuria, by immersing the feet in ice water for about ten minutes.

The Ehrlich test is carried out by ligating one finger of the patient, immersing it in ice water for a few minutes, warming it, then examining the blood below the ligature for hemoglobinemia.

As in the Donath-Landsteiner test, both of these tests may be negative if the patient's complement has been exhausted by previous attacks.

Prognosis.—Paroxysmal hemoglobinuria is not considered fatal, although there is a record of one death during an attack. The attacks may be sufficiently frequent and severe to produce marked clinical anemia. Antisyphilitic treatment will control the attacks generally. There have been no authenticated spontaneous cures.

Treatment.—It is generally accepted that a cure can be obtained by vigorous and prolonged antisyphilitic therapy with arsphenamine, iodides, mercury, and bismuth. Protecting the patient from cold is an important prophylaxis against individual attacks. It has been reported that during treatment the clinical manifestations cease, the Wassermann reaction becomes negative, and the autohemolysin disappears from the serum in the order named.

Many other therapeutic procedures have been recommended, such as autoserotherapy, normal human serum, normal horse serum, cholesterin, calcium, peptone, and sodium chloride. These measures are not rational and have been discarded as being of little value.

CASE 1.—H. D. (No. 66512). White male, aged thirty. University of Virginia Hospital.

Complaint.—Bloody urine following a chill.

Marital History.—Wife receiving antisyphilitic treatment. One spontaneous miscarriage, five children alive and well.

Past History.—Genital sore, diagnosed soft chancre, in 1914. Treated by local applications. No intravenous therapy. Uses alcohol and tobacco freely.

Present Illness.—On January 2, 1928, a bitter cold day, the patient worked hard as a freight handler all morning out of doors. He was tired and thoroughly chilled by lunch time. Immediately after eating a cold lunch he noticed a shaking chill, acute epigastric and substernal cramps, nausea, vertigo, weak-

ness, and the subjective symptoms of fever. In a short while there was an urgent desire to urinate. This urine was deep red in color. After three or four hours of warmth he became comfortable except for a slight headache and malaise. The red urine did not persist. The next morning he went to work feeling about as usual. He had almost identically the same experience that day. He entered the Out-Patient Department on January 4, 1928, bringing with him a specimen of the dark red urine. On spectroscopic examination the color proved to be due to oxyhemoglobin. The chemical and microscopic examinations of this specimen showed it to be normal in all other respects. He had had other similar attacks on two successive days in January, 1927, and on December 1 and 15, 1927. In none of the attacks were any peripheral vasomotor symptoms noted.

In the hospital the following observations were made: The physical examination was essentially negative. The Donath-Landsteiner reaction was negative when first tested but became positive after five days and remained positive throughout one course of eight weekly arsphenamine and bismuth injections. The blood Wassermann was strongly positive. The spinal fluid showed a trace of globulin, 40 lymphocytes per c.mm., and a negative Wassermann. The Rosenbach test produced slight nausea and, according to the patient, prodromes of a paroxysm, but no hemoglobinuria and no hemoglobinemia. The hemoglobin and erythrocytes on January 7 were 47 per cent and 3,200,000 respectively. On January 11 they were 75 per cent and 4,100,000 respectively (each of these observations was checked by a second observer). The blood smear on January 7 showed slight secondary anemia. The icterus index was 2.5 on January 7. On January 11, the day of the Rosenbach test, it was 9. The blood urea, calcium, phosphorous, bleeding time, clotting time, platelet counts, reticulocyte counts, and resistance of erythrocytes to hypotonic saline, were normal. The routine examination of stools and urine, phenolsulphonephthalein and Mosenthal tests of kidney functions were likewise normal.

Subsequent Course.—The patient moved to another city after one course of arsphenamine, bismuth, mercury, and iodides. He was referred to a syphilis clinic in that city but did not appear for treatment. He returned to Charlottesville in about one year. He had no further attacks until January, 1930, when he had one following a long ride in an open car one bitter cold night. The patient has steadfastly refused to report back to the syphilis clinic for further observation and treatment.

SUMMARY AND CONCLUSIONS

A case of paroxysmal hemoglobinuria is reported together with a brief review of the clinical and laboratory findings in this condition.

One wonders if an appreciable number of anemias of late syphilis may not be due to unrecognized abortive, subhemoglobinuric attacks of paroxysmal hemoglobinuria.

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SOME EFFECTS OF AMYTAL ANESTHESIA*

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THE striking announcement by Page[1] that "isoamyl ethyl barbituric acid has been shown to have little or no influence on blood sugar in amounts sufficient to produce surgical anesthesia," apparently so captured the attention of physiologists, that, to judge from physiologic literature since 1923, amytal came at once into almost universal use as an anesthetic for laboratory animals, and this in spite of the scanty knowledge with regard to its effects on physiologic processes. It soon became evident that amytal, like other anesthetics was not without some disadvantages for the study of physiologic processes.

During amytal anesthesia there appears to be little or no effect on blood sugar,[1, 2, 3] on the blood P_H or CO_2 combining power of the blood,[4, 5] or on blood concentration.[6] In spite of Cori's conclusion that the use of amytal is without effect on carbohydrate metabolism,[7] there is evident impairment of glycogenetic function as shown by decreased capacity to assimilate injected glucose;[5] furthermore, in glucose tolerance tests under amytal, the sugar if introduced by the stomach tube does not leave the stomach.[8] Weiss[9] makes the statement that among the main effects of a hypnotic dose of any member of the barbituric acid series is the cessation of peristaltic movements of the gastrointestinal tract. This probably accounts for the failure of sugar to leave the stomach. But even when sugar is injected directly into the duodenum, absorption is impaired, since the blood sugar does not rise as high as in glucose tolerance tests on unanesthetized animals.[8] Although insulin causes uniform decrease in the blood sugar under amytal,[1] the usual rise in respiratory quotient may be delayed or even absent,[3, 10] and although there is typical hyperglycemia following splanchnic stimulation[1] and injection of adrenalin,[11] there is little or no rise in blood sugar after two minutes asphyxiation,[11] or injection of morphine.[12] With large doses there is decrease in metabolism and lowering of body temperature.[4] It is claimed that amytal affects the heart rate, first increasing then decreasing it;[13] Weiss[9] states that with derivatives of barbituric acid the cardiac rate is increased, but in the heart-lung preparation there was no evidence of change in heart rate when amytal was added to the perfused blood.[14] Amytal abolishes, or at least profoundly depresses, the effect of vagus stimulation on the heart, and tends to depress the circulation generally;[15, 16, 17, 18] the initial lowering of blood pressure may, however, be only temporary.[19] In the heart-lung preparation the diastolic volume of the heart is increased and at the same time the output of

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blood is seriously reduced, as long as the amytal remains in the perfused blood.[14] Its effect on the cerebral vessels is to cause dilatation when they are perfused at a constant P_H of 7.6.[20] It lowers the respiratory rate;[13] it immediately stops water diuresis;[21, 22] it does not, however, interfere with rhythmic contractions of the uterus, either in vivo or in vitro;[23] and in contrast to other anesthetics such as ether and chloroform, amytal causes dilatation of the spleen, not contraction.[24]

The ease of administration and the absence of the usual disagreeable after-effects of anesthesia, i.e., nausea, retching and vomiting, have especially recommended it for use with human patients.[25]

RESULTS OF EXPERIMENTS

In all the following experiments the amytal was prepared by adding 1 gm. of amytal to 9 c.c. N/2 NaOH, warming the solution carefully, filtering, and injecting intraperitoneally 0.5 c.c. of the filtrate per kilo body weight. Blood sugar was estimated by the Folin-Wu method.

(1) *Effect on Blood Sugar.*—The majority of observers have confirmed Page's finding that the blood sugar of laboratory animals is not affected by amytal. Weiss,[26] however, found that in dogs and cats there was a sudden rise in blood sugar after injection of amytal either intravenously or intraperitoneally, and upon examining hospital records, we find this is true for human patients. This may be due to the action of the drug on thalamic centers as Weiss suggests, but it may also be related to the amount of carbohydrate (liver glycogen) available for appearance as blood sugar. We found the postabsorptive blood sugar of four dogs before injecting amytal, and then followed the blood sugar at half hour intervals after amytal, first, when the dogs had been fed on ground lean meat for a week, and again after they had been fed for a week on the same daily ration of lean meat with 100 gm. of glucose added. We found no change in the blood sugar when the dogs were on the meat diet alone, but with meat plus sugar there was in each case a very slight rise in blood sugar during the first half hour after amytal, an average in six experiments of 15 mg.; the blood sugar remained at the higher level for about two hours, then returned to the original level.

The figures given by Brill[12] show that morphine injected twenty minutes after amytal causes only a very slight rise in the blood sugar in dogs, 25 mg. within the first half hour (average of seven experiments). This proves to be the maximum, for during the next half hour the blood sugar begins to fall. When morphine alone is given, the blood sugar is increased by over 100 mg. but does not reach its maximum for over an hour, and remains near this maximum for another hour. We have also found that when morphine (10 mg. morphine sulphate per kilo body weight) is injected at the same moment with amytal there is practically no rise in blood sugar and the level remains constant for some six hours. The question rises as to the power of amytal to check the morphine hyperglycemia when the glycogenolysis is at its height, i.e., during the second half hour. We found that amytal caused the blood sugar to fall practically at once.

Amytal therefore not only impairs glycogenesis,[5] but also prevents glycogenolysis. This is further shown in experiments on asphyxiation. We had previously reported that two minutes' asphyxiation of dogs under amytal did not cause a rise in blood sugar.[11] The same is true for the "decapitate cat" preparation. Incidentally, however, we found that amytal did not prevent the rise in blood sugar which follows the operation of decapitation, for there invariably followed a rise of approximately 50 mg., e.g., from 118 to 173, 105 to 161, etc. If, however, asphyxiation is continued for three and one-half minutes until the heart begins appreciably to fail, and heart-block is evident, the blood sugar rises considerably, e.g., from 160 to 222, 170 to 252, etc. This undoubtedly coincides with the liberation of epinephrine in the late stages of asphyxia. We have confirmed Page's[1] statement that splanchnic stimulation during amytal anesthesia produces typical hyperglycemia.

(2) *Effect on Circulation.*—Page[1] states, "Like barbital, iso-amyl-ethyl barbituric acid does not affect blood pressure. Weiss[9] and others, however, claim that the blood pressure does fall slightly. We have obtained the hospital records of ten surgical cases in which amytal was used as an anesthetic. In seven, ¼ gr. morphine sulphate was injected three quarters to three hours before the intravenous injection of 15 gr. 10 per cent "Sodium amytal," and consequently were not of use for our purposes.

In three cases, however, amytal alone was given. In each of these the systolic blood pressure fell: Mrs. S., from 110 to 70, rising in one hour to 85 and in two hours to 100; Mrs. O., from 162 to 100, rising to 120 in three hours; Mrs. M., from 145 to 70, rising in forty minutes to 90 and in four hours to 110. This is the same range of fall as was seen in the seven cases in which morphine was administered first, i.e., 140 to 90, 130 to 90, 105 to 68, etc. In these human patients, therefore, amytal caused a distinct fall in blood pressure.

The effect on heart rate was also followed in two of these patients: Mrs. O., the rate increased from 80 to 110 in the first half hour, returning to 80 in thirty hours; Mrs. M., from 82 to 100, returning to 90 in four hours.

In cats decapitated under ether and two hours later injected intraperitoneally with amytal, we could detect no change in blood pressure. It must be remembered, however, that in decapitate cats the blood pressure is already very low, e.g., 30 mm. When this procedure was repeated on decerebrate cats the lowering of blood pressure was quite pronounced, i.e., from 86 mm. to 44 mm. in less than four minutes; ninety minutes later it was only 56.

We found the effect of splanchnic stimulation on blood pressure in amytalized cats to be fairly normal, e.g., in one case the blood pressure thirty minutes after amytal was 76 mm.; upon splanchnic stimulation it rose to 190; later when it had fallen to 106 mm., a second period of stimulation caused a rise to 182 mm. In another animal one and one-half hours after amytal, the blood pressure rose from 106 to 200 upon splanchnic stimulation; a second dose of amytal was now given intravenously; the heart stopped, but was revived by massage through the chest wall; within fifteen min-

utes the pressure had risen from 9 to 56 mm., and upon splanchnic stimulation promptly climbed to 120 mm.

Cats under amytal, however, did not show the normal effects of asphyxia on blood pressure. We found that in intact cats, and in decapitate and decerebrate preparations with the vagi cut, asphyxiation for three minutes within an hour after amytal caused either no rise or only a very slight, slow rise of some 10 to 20 mm., the most marked feature being the slow fall to 0 as the heart gradually failed. In one decapitate preparation, however, which was asphyxiated more than three hours after injection of amytal, the typical blood pressure curve was shown, the primary and secondary (adrenalin) curves being clearly indicated.

(3) *Effect on the Urea and Nonprotein Nitrogen of the Blood.*—We found in twelve experiments on dogs and in observations on five hospital patients that without exception the urea and nonprotein nitrogen of the blood increase markedly after amytal. The course of the rise is shown in Fig. 1.

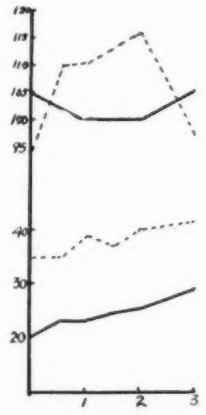

Fig. 1.

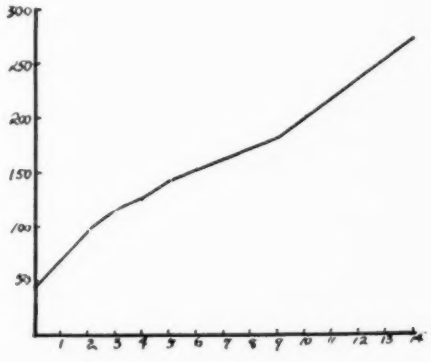

Fig. 2.

Fig. 1.—Effect of lean meat diet (solid line) and meat and sugar (broken line) on blood sugar (upper curves) and on nonprotein nitrogen (lower curves). Abscissae, time in hours after amytal.

Fig. 2.—Daily increase in blood urea in man with both kidneys removed surgically.

We have followed the blood urea of one human patient both of whose kidneys were removed surgically. There was no infection, since the leucocyte count remained at 7,900, and the patient's temperature remained normal until just before death. The blood urea before the operation was 47 mg. Fig. 2 shows the increase in urea from the time of operation until death exactly two weeks later. The data show that an average of 0.7 mg. urea per 100 c.c. of blood was added to the blood hourly. Crowden and Ogden (private communication) found in cats deprived of both kidneys an hourly increase of 4 mg. urea. In our twelve experiments on dogs we found that during the first two hours after amytal the average increase in urea was 2.6 mg., and in nonprotein nitrogen 2.3 mg. Further work is in progress to determine whether this is the result of amytal acting directly on the kidney, or merely the result of depressed circulation.

(4) *Effect on Respiration.*—The slowing of respiration under amytal has been noted by many observers, and Weiss[9] notes that Cheyne-Stokes' breath-

ing may occur. We have tested the effect of amytal on respiration by placing dogs in a specially constructed tank (to be described elsewhere) in which the air pressure could be lowered to any desired degree, yet fresh air at the given pressure constantly entered at one side of the chamber. This fresh air was set in motion by means of an electric fan, and was constantly carried away at the other side. In this tank rats have been kept alive for months at less than half an atmosphere pressure. We found that a normal dog in this tank began to show evident respiratory effects when the pressure was reduced to 560 mm. At this pressure there was no change in rate, but the depth of inspiration became decidedly greater as shown by the greater movement of the flanks. In general this increase in depth of respiration took place at about 550 mm., but in some dogs it did not occur until 460 mm. At 460 mm. there was usually an increase in rate as well as depth. Schneider[27] found that in general man shows the first definite respiratory response at 16 to 14 per cent O_2, approximately 570 mm. pressure, by an increase in lung ventilation, but that increase in rate does not make its appearance until the O_2 is lowered to 8 or 6 per cent, 250 mm. pressure. There were usually evident signs of distress in our dogs at 400 mm., for they became uneasy, stood up if they had been previously lying down, and sometimes scratched at the sides of the tank. The following record is a fair sample and in this case increase in rate of respiration occurred in the amytalized dog at a slightly less reduced pressure than when the dog was unanesthetized:

11:00 A.M.	Dog in tank apparently asleep; 10 breaths in 36, 40, 39, 45, 46 sec.
11:19 A.M.	Pressure lowered.
11:26 A.M.	Pressure 600 mm.
11:29 A.M.	10 breaths in 44 sec.
11:30 A.M.	Pressure lowered to 560 mm.
11:31 A.M.	10 breaths in 43 sec.; definitely deeper.
11:35 A.M.	10 breaths in 43 sec.
11:35½ A.M.	Pressure lowered to 500 mm.
11:37 A.M.	10 breaths in 42 sec.
11:39 A.M.	Pressure lowered to 435 mm.; much deeper.
11:40 A.M.	10 breaths in 26 sec.; dog moved.
11:41 A.M.	10 breaths in 23 sec.
11:41½ A.M.	Pressure increased gradually.
11:48 A.M.	Atmospheric pressure; 2 c.c. amytal injected intraperitoneally.
11:59 A.M.	Dog well under; 10 breaths in 34 sec.
12:00 M.	10 breaths in 38, 37, 40 sec.
12:05 P.M.	10 breaths in 45, 45 sec.
12:07 P.M.	10 breaths in 45 sec.
12:09 P.M.	10 breaths in 45 sec.
12:10 P.M.	Pressure gradually lowered.
12:14 P.M.	Pressure 500, 10 breaths in 28 sec.
12:16 P.M.	10 breaths in 28 sec.

The procedure was repeated with practically identical results.

In another amytalized dog, reducing the pressure to 580 mm. was sufficient to increase the rate from 19 breaths per minute to 30. This was repeated several times with the same results. In both these cases the body

temperature did not fall and the rate of respiration after amytal was the same as before amytal. In another case, however, no change in rate was observed even at 460 mm., nor was there any change in depth, though when unanesthetized this same dog showed deeper respirations at 560 mm. In several dogs the respiratory center was very strongly depressed and in these cases the body temperature fell as low as 35.5°. In one of these dogs the rate before amytal was 30 breaths per minute at 760 mm., increasing to 40 at 500 mm., but after amytal there were only 4 breaths per minute at 760 mm., 5 at 500 mm., 10 at 400 mm., and 16 at 300 mm.; each breath, no matter what the pressure, was very deep and involved vigorous movement of the flanks.

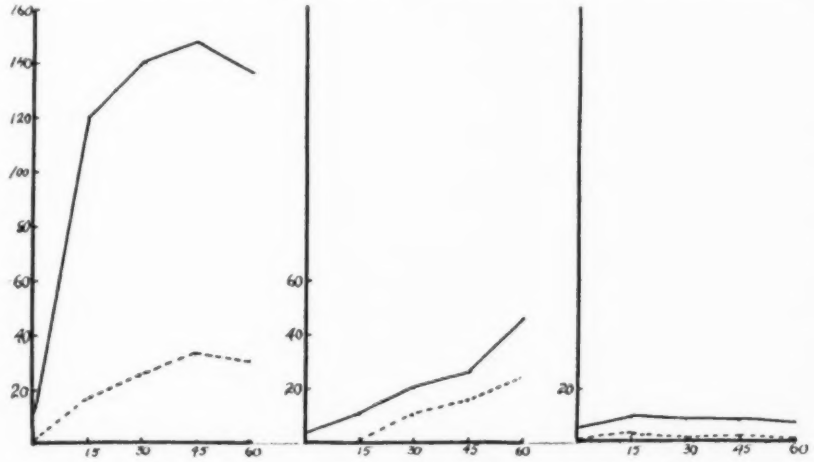

Fig. 3. Fig. 4. Fig. 5.

Figs. 3, 4, 5.—Gastric secretion after alcohol test meal. Total acid, solid line; free HCl, broken line. Abscissae, time in minutes after test meal; ordinates, c.c. N/10 HCl. Fig. 3, perfectly trained dog; Fig. 4, restless dog; Fig. 5, same dog as in Fig. 3 under amytal.

The effect of amytal on the respiratory center of decerebrate cats proved to be very striking. The following record is typical:

Cat decerebrated under ether 10 A.M.

1:30 P.M.	14 breaths per min.	
1:31 P.M.	15 breaths per min.	
1:32 P.M.	15 breaths per min.	
1:33 P.M.	16 breaths per min.	
1:34 P.M.	15 breaths per min.	Amytal now injected.
1:35 P.M.	15 breaths per min.	
1:36 P.M.	15 breaths per min.	
1:37 P.M.	14 breaths per min.	Definitely shallower.
1:38 P.M.	13 breaths per min.	
1:39 P.M.	12 breaths per min.	
1:40 P.M.	12 breaths per min.	Very shallow; difficult to detect.
1:43 P.M.	13 breaths per min.	Less shallow.

In several cases when the respirations were recorded on a smoked drum, about five minutes after amytal, the respirations became so shallow that they failed to record. In one case there was no visible movement for thirteen minutes, after which breathing took on the Cheyne-Stokes' character, but became quite regular, though very deep, in another ten minutes.

Amytal seems to have a profound effect on the respiratory center, but the details of behavior vary from animal to animal.

(5) *Effect on Gastric Secretion.*—For this purpose we used the alcohol test meal.[28] Fifty c.c. of 7 per cent ethyl alcohol plus 1 c.c. of 0.5 per cent phenolphthalein were introduced into the stomach by tube. One hundred c.c. H_2O were then added. Within three to five minutes 2 c.c. of the stomach contents were withdrawn; to 1 c.c. was added excess of NaOH to serve as a standard for the depth of phenolphthalein color; the other was used for estimation of free HCl and total acidity. Similar samples were withdrawn at fifteen-minute intervals. In a quiet, trained dog (unanesthetized), the rise in free HCl and total acidity is marked (Fig. 3), and even in a restless dog constantly struggling a certain degree of gastric secretion takes place (Fig. 4) (cf. the results of Hartzell[29] in this respect). In five experiments in which the test was applied to the same dogs unanesthetized and when under amytal, it was found that there was no secretion of free HCl whatever within the hour after injection of amytal (Fig. 5); in four of the five trials the total acidity remained constant and in the fifth it increased by only 12 c.c. within the hour. In all five cases there was little or no change in the phenolphthalein color, showing that there was no dilution of the dye and therefore there was no change in the volume of the gastric contents, a further proof of lack of gastric secretion.

SUMMARY

1. Dogs fed on a diet rich in carbohydrate show a slight rise in blood sugar after amytal. Dogs on a diet of lean meat only showed no such rise.

2. Amytal not only prevents hyperglycemia when injected simultaneously with morphine, but also checks glycogenolysis if injected later than the morphine.

3. Amytal prevents the rise in blood sugar which would normally follow two minutes asphyxia, but does not prevent it in the very late stages of asphyxia.

4. In the human patients under our observation the blood pressure fell after amytal and the heart rate increased. The blood pressure in decerebrate cats invariably fell after amytal.

5. The effect of splanchnic stimulation in cats under amytal appeared normal.

6. Asphyxia failed to cause the typical rise in blood pressure in cats under amytal.

7. The urea and nonprotein nitrogen of the blood increase to a greater degree after amytal than in individuals with both kidneys removed. This is probably due to depressed circulation.

8. Amytal profoundly affects the respiratory center.

9. Amytal prevents gastric secretion.

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THE LEUCOCYTIC REACTION TO INJECTIONS OF A NONSPECIFIC PROTEIN*

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THE literature contains many references to the use of nonspecific protein in the treatment of infections and infective processes. These deal principally with its value in the presence of such lesions as gonorrhea and its complications, chronic arthritis, certain dermatologic lesions, abscess formations, and other conditions of an infective nature.

At first, the agent used for this form of therapy was a vaccine, most often a typhoid vaccine. Later, boiled, skimmed milk was utilized. Then followed special pharmaceutical preparations of milk, defatted, sterilized and put up in ampule form, ready for injection. Our work was done with a preparation of the latter type.

The mode of action of an injected foreign protein is speculative and uncertain. It is thought that the "shock" produced by it causes, in some way, a salutary reaction on the part of the body tissues against infection. It is claimed by all the exploiters of preparations for nonspecific protein therapy that its administration is followed by an increase in leucocytes and antibodies. Antibodies are hypothetic and illusive creatures which cannot be captured or counted, but leucocytic reaction may be definitely measured.

One of us (R. L. L.) has had a fairly large experience with the use of nonspecific protein therapy in a variety of infective lesions and has been impressed with its value. Wishing to learn more, if possible, of its mode of action, it was decided to test the claim that leucocytosis results from such therapy. An accurate measurement of the leucocytic reaction is a feasible procedure and, if the claim could be substantiated, this phenomenon might explain, at least in part, the virtue of an injected protein in the treatment of disease. With this in mind, the aid of the co-author (A. A. J.) was enlisted and subjects procured.

The protein preparation used was Proteolac (Searle) which is described as "defatted milk which has been subjected to a digestive process in the laboratory which converts it into a clear, amber-colored solution of proteoses and peptones." It was claimed by Vaughan[1] that these split proteins, proteoses and peptones, are the active bodies in protein therapy and this is the reason given by the manufacturers of this product for presenting it in this form.

Twelve apparently healthy individuals were obtained, most of them through an employment agency. Immediately following an initial leucocyte count, each of these was given his first injection of Proteolac. The succeeding injections were given twice a week at three and four day intervals. Four, eight, and twenty-four hours after each injection, further counts were made.

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‡Director of the North Shore Clinical Laboratory.

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Four different types of injection were given: (a) 3 to 5 minims intradermally; (b) 1 c.c. intramuscularly; (c) 5 c.c. intramuscularly, and (d) 10 c.c. intramuscularly. For the second injection, only five of the patients were available, the others having obtained employment, and this was reduced to four for the subsequent injections. None of the patients complained of chills, fever, pain or other untoward reactions and all said that they would have been glad to go on with the experiment if they had not obtained regular employment.

Table I shows the leucocyte counts obtained before and after the first injection:

TABLE I

SUBJECT	SEX	AGE	INJECTION	LEUCOCYTE COUNTS			
				BEFORE INJECTION	4 HOURS AFTER INJ.	8 HOURS AFTER INJ.	24 HOURS AFTER INJ.
1	F	30	3-5 min. intraderm.	5650	6050	5200	5250
2	M	40	1 c.c. intramusc.	4700	8700	10000	8100
3	F	36	1 c.c. intramusc.	5700	4800	5850	5100
4	M	19	5 c.c. intramusc.	7350	7150	8800	7200
5	M	53	5 c.c. intramusc.	4000	4650	7800	4100
6	M	26	1 c.c. intramusc.	7800	8450	9900	8400
7	M	26	10 c.c. intramusc.	4800	3950	8100	7200
8	M	24	10 c.c. intramusc.	6100	6850	7700	4400
9	M	29	10 c.c. intramusc.	8500	6050	7050	6100
10	F	28	5 c.c. intramusc.	8900	5200	6200	6200
11	M	12	3-5 min. intraderm.	7000	5200	6800	7000
12	F	8	3-5 min. intraderm.	6600	5400	6800	6600
Averages				6425	6038	7517	6304

If any conclusion may be drawn from the above presentation, it is that one injection *only* of a nonspecific protein must be valueless. Table I indicates that, with a few exceptions, the first injection is followed by a drop of the leucocyte count in the first few hours, followed in the next few hours by a slight rise above the normal and a return to approximately normal in twenty-four hours. Also, the initial counts are substantially in agreement with the report of Garrey and Butler[2] that the normal leucocyte counts in healthy persons average between 5000 and 6000 rather than between 7000 and 8000 as is usually thought. Six of our patients gave counts of from 4000 to 6100, previous to injection; four gave counts between 6600 and 7800, and two showed more than 8000. Nine gave counts of 7000 or less while only three showed more than 7000.

Table II gives a résumé of the findings in the four patients who received the full series of six injections of Proteolac.

The four patients in Table II correspond in number with those of Table I. It will be noted that the types of injection were changed after the first dose in

TABLE II

RÉSUMÉ OF RESULTS FROM FOUR PATIENTS WHO RECEIVED A SERIES OF SIX INJECTIONS OF PROTEOLAC

SUBJECT	SEX	AGE	TYPE OF INJECTION	SERIES	LEUCOCYTE COUNTS					
					BEFORE INJECTION	4 HOURS AFTER	8 HOURS AFTER	24 HOURS AFTER	PER CENT INCREASE	LATER COUNT
1	F	30	3-5 min. intraderm.	1	5650	6950	5200	5250		7800
				2		7450	7200	7150		(23 days after last injection)
				3		7200	7800	7700		
				4		7100	8050	7900		
				5		7700	8100	7700		
				6		7900	9900	8700	54	
Averages						7383	7708	7400		
2	M	40	1 c.c. intramusc.	1	4700	8700	10000	8100		8200
				2		?	8450	11650		(23 days after last injection)
				3		9100	10200	9700		
				4		12100	9900	9950		
				5		9900	?	8100		
				6		?	10200	10100	115	
Averages						9950	9750	9600		
3	F	36	5 c.c. intramusc.	1	5700	4800	5850	5100		8200
				2		6000	6800	6400		(33 days after last injection)
				3		5600	7100	7600		
				4		6900	7700	7300		
				5		6950	7900	7300		
				6		7250	7950	7900	39	
Averages						6250	7217	6933		
4	M	19	10 c.c. intramusc.	1	7350	7150	8800	7200		
				2		9450	14350	7550		
				3		8050	11200	8100		
				4		9100	15100	9000		
				5		9100	10800	9850		
				6		10800	17050	11200	52	
Averages						8942	12883	8817		
Grand Averages					5850	8131	9389	8188	65	

patients 3 and 4. These were given 1 c.c. and 5 c.c. intramuscularly respectively for the first dose and 5 c.c. and 10 c.c. respectively for all subsequent doses, in order to make it possible to continue the series with injections of each type with the four individuals who were available for the entire series.

Charts 2, 3, and 4 show the individual curves of leucocytic reaction in the four subjects. Chart 1 shows composite and mean curves of leucocytic reaction in all the patients.

It will be seen, on analysis of the data, that in each patient the injections were followed by steadily rising leucocyte counts. In patients 1, 3 and 4, the

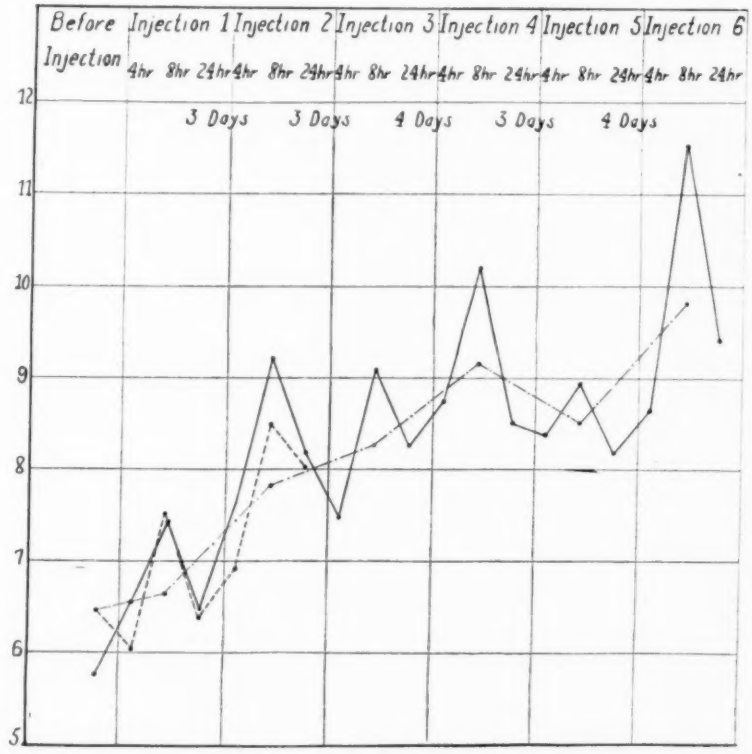

Chart 1.—Composite curves of leucocytic reaction. The heavy line is the composite curve of the four individuals who received six injections. The dotted line is the composite curve of the twelve individuals who received but one injection and five who received two injections. Note how closely the curves approximate each other. The broken line is the mean curve of reaction for the series.

The numbers on the left margin indicate the leucocyte counts in thousands.

counts receded after the first injection to levels which were lower than the initial counts but these levels were raised after each succeeding injection, almost without interruption, to successively higher points. Also, in patients 1, 3 and 4, it will be noted that the highest leucocyte counts were most often obtained eight hours after injection while the twenty-four hour counts usually receded to approximately the same levels as the four hour counts. In patient 2, the highest counts were, as a rule, found four hours after injection and these were followed by recessions to approximately the same figures for the eight and twenty-four hour counts.

The percentage increases in Table II are based on the final counts, twenty-four hours after the last injection, as compared with the original counts before any injection had been given. It is interesting here to note that the greatest percentage increase was found in patient 2 who received but 1 c.c. doses of Proteolac intramuscularly and the next highest was found in patient 1 who

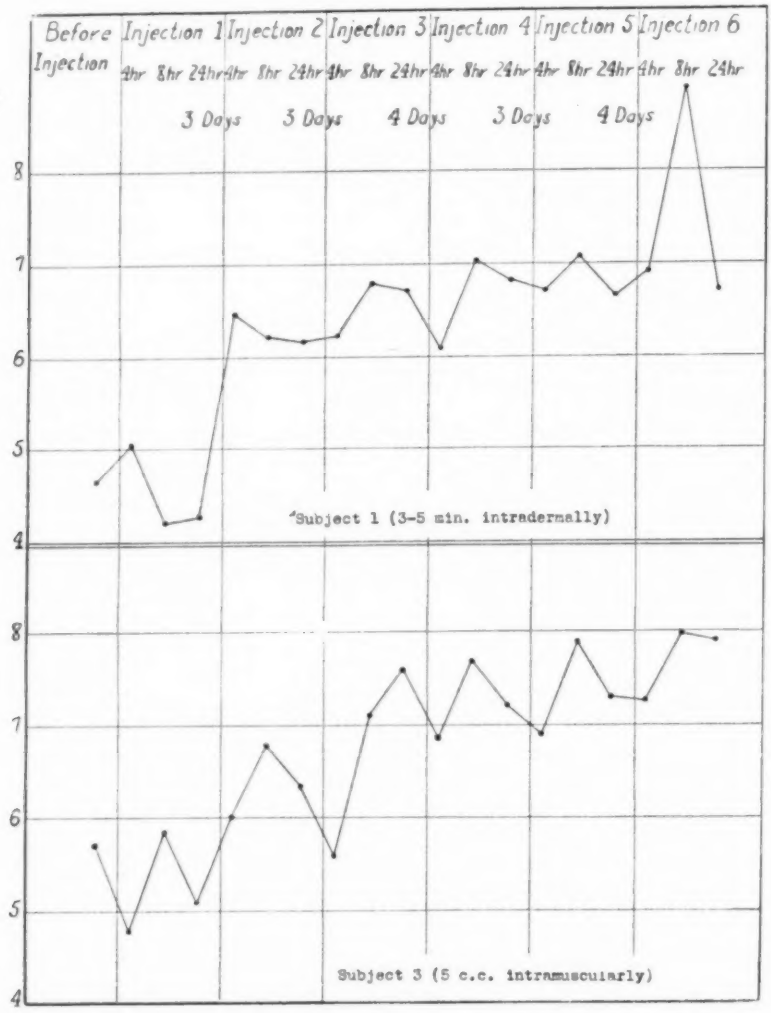

Chart 2.—Leucocytic reactions of patients 1 and 3. The figures on the left margin indicate the leucocyte counts in thousands.

received doses of only 3 to 5 minims intradermally. In such a small series it is not possible to draw definite conclusions but such reactions would seem to indicate that the *size* of the dose *may* not be an important factor.

DISCUSSION

None of the subjects of this experiment complained of any local reaction beyond experiencing a little temporary tenderness in the muscles injected which

did not interfere with their actions in any way and did not deter them from returning. Not one had any general reaction with rise in temperature, chills or other symptoms. This brings up the question of whether such a general reaction is necessary or important in obtaining the desired results when non-specific protein therapy is used.

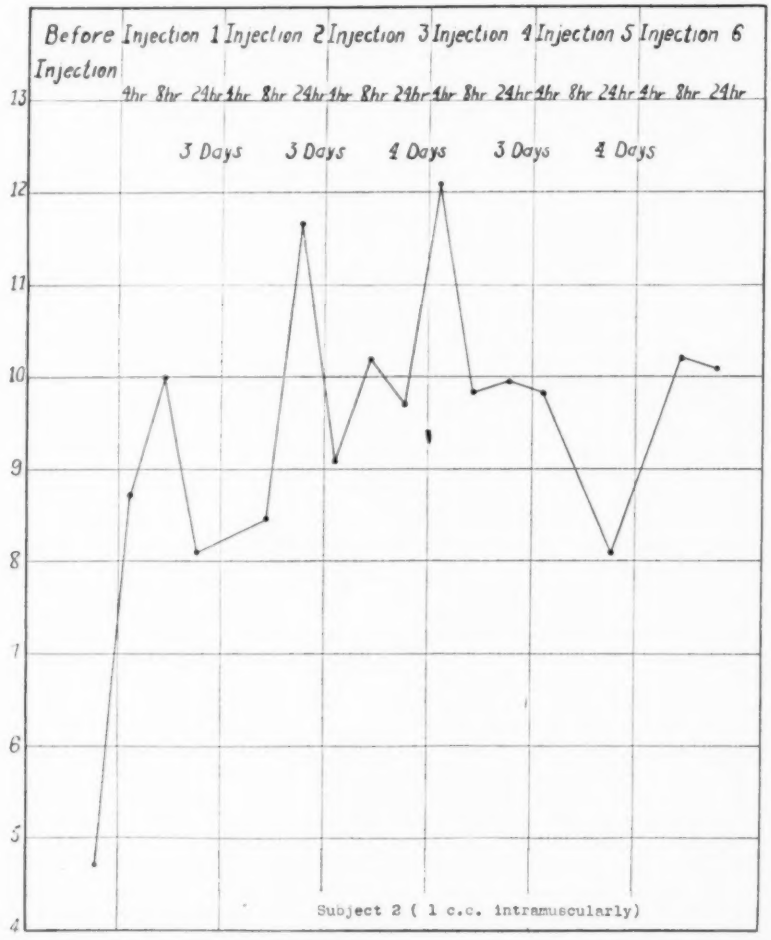

Chart 3.—Leucocytic reaction of patient 2. The figures on the left margin indicate the leucocyte counts in thousands.

Many physicians believe that a "shock" with chills, fever, etc., is essential to success while others feel that it is not. In our own work, we have noted excellent results in many cases from using preparations which were not attended by such reactions; and our recorded figures show that, if an increase in leucocytes is an indication of growing resistance against infection, a general reaction is not necessary to increase this resistance. A definite increase in leucocytes was obtained by the use of a preparation which did not cause any general symptomatic reaction whatever.

How long the increase persists after cessation of administration was not determined, but the last column of Table II shows that several weeks after the final injection the leucocyte counts of three of the subjects were considerably higher than their initial counts.

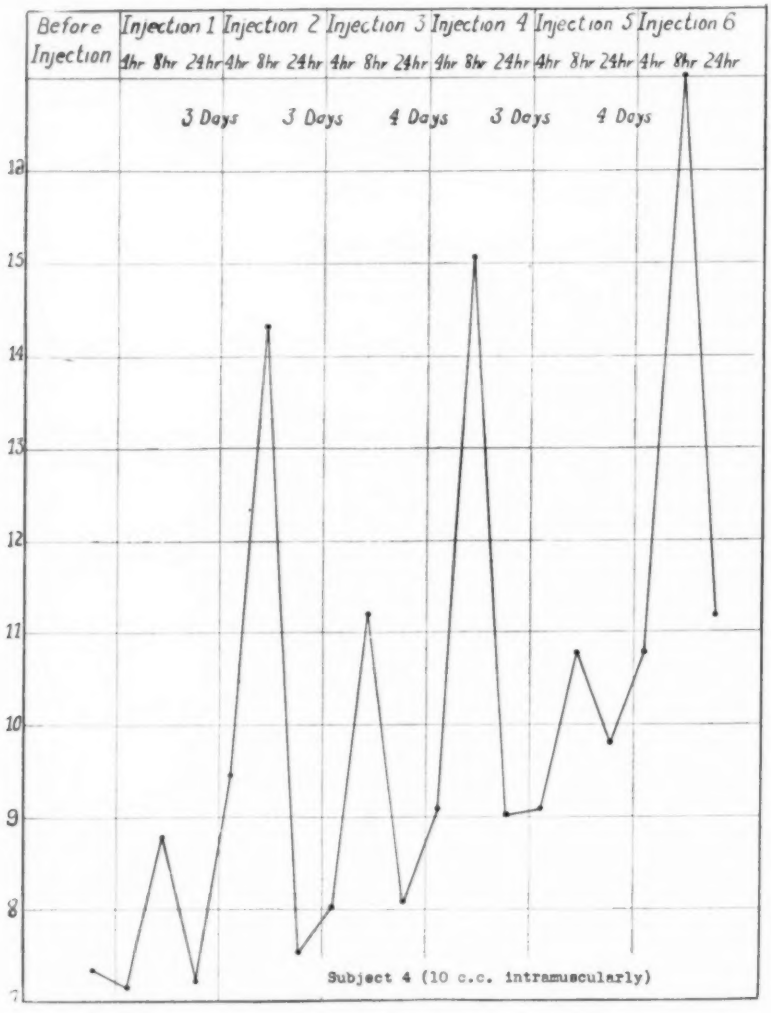

Chart 4.—Leucocytic reaction of patient 4. The figures on the left margin indicate the leucocyte counts in thousands.

CONCLUSIONS

A definite, persistent and progressive increase in leucocytes was obtained in four individuals who were given six injections of Proteolac, a nonspecific protein preparation. An evanescent increase was also observed in twelve individuals who were given only one injection.

The question is raised as to whether a general "shock" reaction is essential to success with this form of therapy. If the leucocytic increase is a measure

of resistance to infection, such a reaction is not essential, because the increase can be obtained without a symptomatic "shock."

In our small series, the leucocytic reaction (percentage increase) was greater in the subjects receiving small doses intradermally and intramuscularly than in those receiving larger doses intramuscularly, and suggests that the *size* of the dose *may* not be an important factor.

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636 CHURCH STREET.

THE INFLUENCE OF ANTICLOTTING AGENTS ON TRANSPLANTATION AND GROWTH OF TUMOR TISSUE*

BY ALFRED GOERNER, M.D., LONG ISLAND, N. Y.

THE effect of various substances on the transplantation of tumors in vivo and on cultures of tumor tissue in vitro has been studied by numerous workers. Lewin[1] found that sodium nucleinate inhibited the transplantation of tumor tissue as did also some protein derivatives. Blumenfeld[2] observed that blood drawn from the vein of a tumor would give a positive transplant in a much greater number of cases when the blood was allowed to clot, whereas the addition of sufficient citrate to prevent clotting gave mostly negative results. Cramer[3] showed that the exposure of cancer cells to relatively large amounts of calcium decreased their capacity for growth when inoculated. Goerner and Shafiroff[4] have shown that small amounts of calcium increase in the circulating blood will enhance tumor growth in animals.

It is interesting to note that the substances mentioned above all possess the power of preventing the clotting of blood. In the case of calcium small amounts enhance clotting while larger quantities prevent this. This is, as far as the author can ascertain, the first correlation between clotting mechanism and tumor transplantation and growth.

In view of the fact that irritation and injury play an important rôle in the genesis of malignant tumors and also because Heilbrunn[5] pointed out the analogous processes underlying cellular injury and blood clotting, it seemed interesting to try the effect of other known anticoagulants on tumor transplantation and growth. It is, perhaps, significant in connection with one of these agents (heparin) derived from the liver, that Fischer[6] observed the reluctance with which tumor cells grow in vitro in the presence of liver tissue or extracts.

*From the Department of Biochemistry, The Long Island College Hospital.
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EXPERIMENTAL

Fresh nonnecrotic tumor tissue of the Flexner-Jobling rat carcinoma was cut up into small fragments under aseptic conditions. The pieces were made of the same size as near as this was possible. A number of fragments were placed into each of the sterilized solutions of the agents to be tested and incubated at body temperature for various lengths of time. They were then transplanted into white rats and the animals were observed for tumor growth and where this occurred the size was charted. The solutions used were: a 0.1 per cent sodium oxalate in 0.9 per cent sodium chloride, a 0.1 per cent heparin in 0.9 per cent sodium chloride, and a 0.9 per cent sodium chloride as a control. The time of incubation varied from ten minutes to twenty-two hours. It is easily shown that tumor tissue can be transplanted successfully for twenty-four hours or more after removal from the host.

In all cases the fragments for any series were from the same tumor. The animals that showed no positive transplants after three months' observation were inoculated with fresh untreated tumor and positive transplants were observed in all. This showed that the animals were not resistant to the tumor. All animals were of the same breed used by the author in other experiments and have shown 100 per cent positive transplants.

RESULTS

In a series of eighteen animals it was found that there was no appreciable difference in the growth of the tumor from the fragments that had been treated as compared with the sodium chloride control, all having been treated for ten minutes. Each group was made up of six animals. The relative growth can be seen from the condensed Chart I. Only one animal has been taken from each group of six to illustrate this. All animals of this group died or were killed and autopsied.

A very large tumor presenting the typical characteristics of Flexner rat carcinoma was found in each case.

Another series of eighteen animals was divided into three groups of six and inoculated with fragments that had been incubated for three hours in contact with the respective solutions. Here it was observed that the sodium oxalate and the heparin exerted a slight but easily noticed inhibitory effect on the subsequent growth of the fragment of tumor tissue. Chart 2 illustrates this. All of the animals died or were killed and autopsied, showing characteristic Flexner rat carcinoma.

Chart 3 shows the effect of the anticlotting agents as compared with the control after treating the tumor fragments with each solution for twenty-two hours. Six animals were used for each group. The chart shows the results in the case of one animal of each group. The control showed positive transplants in every animal. The tumor was somewhat smaller than in the previous controls treated for ten minutes and three hours but the rate of growth was such that at the end of three weeks after transplantation the controls showed no marked difference in size from the previous controls. In the case of sodium oxalate, however, no tumor was palpable for three and a half weeks after transplantation and the rate of growth was diminished as compared with the

control throughout the period of observation. All of these animals showed a typical Flexner rat carcinoma. In the case of the fragments treated with heparin all of the animals showed no tumors at the end of two or three months' observation. Autopsy showed no trace of tumor tissue at the point of transplantation and none was found in any of the organs. To further prove this point, another series of eighteen animals were inoculated with tumor fragments

Chart 1.

Tumor tissue in contact for 10 minutes

Date	Sodium Chloride	Sodium Oxalate	Heparin
10/19/29	Transplanted	Transplanted	Transplanted
10/31/29			
11/6/29			

Chart 2.

Tumor tissue in contact for 3 hours.

DATE	Sodium Chloride	Sodium Oxalate	Heparin
10/19/29	Transplanted	Transplanted	Transplanted
10/31/29			
11/6/29			
11/11/29			

Chart 3.

Tumor Tissue in contact for 22 hours.

Date	Sodium Chloride	Sodium Oxalate	Heparin
10/19/29	Transplanted	Transplanted	Transplanted
10/31/29			
11/6/29		—	—
11/08/29			—
11/18/29			—
11/25/29	Tumor very much larger		—

kept for twenty hours in each of the solutions mentioned. The results were the same. In this series the animals receiving the fragments of tumor treated with heparin showed no positive transplants, and at the end of three months' observation all six animals were inoculated with fresh tumor tissue. All showed positive transplants indicating that the animals were not resistant. It may be added that the solutions used were made up to P_H 7.0.

RÉSUMÉ

Fragments of tumor of the Flexner-Jobling rat carcinoma type were treated for various lengths of time with sodium oxalate and heparin and then

transplanted into animals. Twenty hours' contact between the anticoagulant and the tumor showed an inhibiting effect in the case of oxalate solution. Heparin prevented the growth of all transplants after similar treatment.

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FATAL ANAPHYLAXIS IN MAN*

By W. M. Sheppe, M.D., Wheeling, West Virginia

SUDDEN death following the injection of foreign protein in man is fortunately very unusual. When one considers the enormous number of such injections being made daily, the actual rarity of such tragedies at once becomes apparent. A careful review of the literature to date indicates that approximately fifty such fatalities have been reported. The exact number of cases cannot be accurately determined, as some reports do not furnish sufficient data for analysis. A certain percentage of the recorded deaths occurred in individuals suffering from serious disease, such as general infections or severe injuries. In such cases sudden death is not uncommon, and the administration of serum may have been coincidental rather than causative. On the other hand, some deaths have no doubt occurred which have not reached the literature. A total of fifteen cases with autopsy have been reported with sufficient completeness to indicate that the train of symptoms ending in death was instigated by the administration of foreign protein. An additional fatal case is herewith described. The findings at autopsy are discussed and certain conclusions drawn as to the mechanism of the reaction.

W. S., black male, aged thirty years, was admitted to the out-patient department of the University of Virginia hospital complaining of a bullet wound in the left shoulder. This wound had been received twenty-four hours before admission. It was a perforating wound of the left deltoid muscle, the bullet having passed downward and backward. A small piece of cloth was carried in with the bullet. The patient received fifteen hundred units of tetanus antitoxin in the subcutaneous tissue of the left lower abdomen. He walked to the cashier's window, paid the fee charged for the antitoxin, left the building, and entered an automobile. After traveling about a quarter of a mile, the patient complained of severe substernal pain and a feeling of breathlessness and marked anxiety. He was immediately returned to the hospital but died before he could be put to bed. The total time elapsing between the injection of the antitoxin and subsequent death was approximately twenty minutes. There was marked dyspnea but very slight cyanosis before death. The dyspnea became progressively more marked. Death occurred before adrenalin could be administered.

*From the Medical Division of the Wheeling Clinic.
Received for publication, July 18, 1930.

POSTMORTEM FINDINGS

The autopsy protocol was recorded as follows:

External Appearance.—The body was that of a well nourished man about thirty years old. There were no abnormal depressions or protuberances about the head. The sclerotics were not tinged. The ears and nose were negative. The teeth were excellent, and the buccal mucosa was normal in appearance. There was a small, deep, lacerated wound about one-half inch in diameter on the left shoulder, just anterior and a little below the head of the humerus. The abdomen was flat. The epitrochlear and inguinal glands were not enlarged. The external genitalia were negative. There were no scars on shins or edema of the feet and ankles. No malformations or deformities were noted. The cervical glands were not enlarged. The axillary groups of glands on both sides were enlarged. The veins of the head and neck were congested, giving a dusky, mottled appearance to that region.

Abdominal Cavity.—The abdominal muscles were well developed. The superficial fat was normal in amount and color. A small puncture wound passing into the abdominal wall was noted about midway between the umbilicus and the anterior superior spine of the ilium. A small hematoma surrounded the path of the puncture as it passed through the subcutaneous tissue. The peritoneum was smooth and glistening, and the puncture wound did not penetrate into the peritoneal cavity. There were no adhesions in the region of the spleen, sigmoid, or gall bladder.

The appendix was congested and turned back on itself, the tip being firmly attached to the meso-appendix by old adhesions. The omentum lay free in the peritoneal cavity and was normal in appearance. The bladder was distended. The abdominal viscera were normal on palpation.

Heart.—The heart was normal in size and shape. The epicardium was smooth and glistening. The myocardium was normal in thickness, color, and consistency. The valves were clear without sclerotic patches or vegetations. The endocardium was smooth and glistening. The coronary vessels were clear. The pericardium was smooth and glistening. It contained about 10 c.c. of clear serous fluid. The right heart, superior and inferior venae cavae, were engorged with blood. The thymus gland was atrophic.

Thoracic Cavity—

1. The veins of the neck and thorax were markedly congested.

2. There was no free fluid in the pleural cavities.

3. The lungs were very large and voluminous and seemed to over-fill the thoracic cavity. The cardiac incisura had almost disappeared, and the middle border of the lungs overlapped in the superior portion of the anterior mediastinum. After the anterior thoracic wall was lifted away, the lungs appeared to bulge forward. There was no evidence of collapse of the lungs when the pleura was opened. The middle lobe of the right lung was adherent posteriorly to the thoracic wall. The pleura of that side was otherwise smooth and glistening. In the posterior axillary line on the left side a hematoma lying outside the parietal pleura was noted. It was about two inches in diameter. A similar hematoma was noted in the latissimus dorsi of that side. The muscle was dissected back and a bullet, apparently of .38 caliber, was found just under the angle of the scapula. There was no fracture of the humerus, ribs, or scapula, nor was there any injury to the axillary or brachial vessels.

Lungs—

1. The lungs were very large and voluminous.

2. They were a very light gray in color.

3. Numerous elevations and depressions were noted on the surface of the lungs, but in general the surfaces were much smoother than the lung surface usually seen at autopsy.

4. Emphysematous areas were seen around the borders of both lungs.

5. When the lungs were removed and placed in a pan of water, they float high in the water as if made of cork. Severance of bronchi did not lead to collapse of the lung. Moreover small blocks cut from the lung and placed on the surface of the water, floated as the whole lung did. In fact, the air can be expressed from small pieces of lung tissue with difficulty. The cut surface was a pale gray in color and was very dry. No gross lesions were noted. There was very slight exudation of blood or serum from the bronchi or blood vessels.

Stomach.—The viscus was dilated with a large amount of undigested food. The mucosa was smooth and glistening. There was no evidence of malignancy or obstruction at the pylorus. The intestines were normal on inspection and palpation.

Liver.—The liver was normal in size, shape, color, and consistency. The gall bladder was full of bile but no stones were palpable in the bladder or the common duct. On section the liver was engorged with blood but was otherwise normal. No tubercles or gummas were noted.

Pancreas.—Normal.

Spleen.—Normal in size, shape, color, and consistency. On section the malpighian bodies stood out distinctly. There were no infarcts or other pathology. The capsule was not thickened.

Kidneys.—Normal in size, shape, color, and consistency. The capsule stripped back with ease. The glomeruli stood out plainly from a cortex which was normal in thickness. The pelvis was of normal size and was free of pus and blood.

Adrenals and Ureters.—Normal.

Bladder.—Was dilated with urine. The wall was not thickened. The mucosa was smooth and glistening. No diverticula nor trabeculations were noted. The prostate was normal in size and consistency. The retroperitoneal and mediastinal glands were not enlarged. The aorta showed no pathology.

Brain and Meninges.—The dura was not under increased pressure. The veins of the dura and the venous sinuses were not engorged. The meninges were smooth and glistening without adhesions. The surface of the brain showed no evidence of trauma or inflammation. It was normal in consistency and color. On section the middle meningeal and cerebral arteries showed no pathology. The cut surface of the brain was normal in every respect. The cerebellum and medulla showed no pathology. There was no evidence of fracture of the vault or base of the skull.

Microscopic Examination.—The sections of lung showed a marked dilatation of the alveoli comparable to that noted in senile emphysema. Some of the alveolar walls were ruptured. No conclusions could be drawn as to the bronchial musculature. There was a notable lack of leucocytic infiltration.

Sections from other organs.—Unimportant.

Anatomic Diagnosis.—Emphysema of lungs, bilateral; congestion of vessels of head and neck; lacerated wound of shoulder, left; hematoma, thoracic wall, left; appendicitis, chronic; pleurisy, chronic fibrous, left; foreign body (bullet) in latissimus dorsi muscle, left; puncture wound of abdominal wall.

It was concluded that death was probably due to an "accelerated" or "guinea pig" type of reaction following the administration of tetanus antitoxin. It was later learned that the patient was an ex-service man who had been wounded in France and had presumably been given tetanus antitoxin at that time. The allergic history was negative.

This case seems to be of particular interest for several reasons. Death occurred in a presumably nonallergic individual who possibly had been sensitized to horse serum by a previous injection. The autopsy findings were definite and were uncomplicated by other morbid conditions. The examination was conducted immediately after death.

DISCUSSION

It is important to report all cases of sudden and unexpected death following the injection of foreign protein. If however no autopsy is obtained, the data both as to the actual cause and the mechanism of death, are of questionable value. All clinicians are familiar with examples of death following some minor trauma, light anesthesia, or emotional strain. Many of these cases

reveal at autopsy an entirely unexpected train of events, so that sudden death in critically ill patients cannot always be attributed to some event such as the injection of serum occurring just prior to death. Boughton[2] comments on the rarity of reported fatalities in vigorous adults and particularly the rarity of autopsies in such uncomplicated cases. This type of death is more likely to be encountered in those dealing with large numbers of allergic patients.[4, 8]

For the foregoing reasons all cases except those containing postmortem data have been eliminated from this discussion.

SUMMARY OF AUTOPSY FINDINGS IN INDIVIDUALS DYING SHORTLY AFTER THE INJECTION OF FOREIGN PROTEIN

A, DEATH FOLLOWING PRIMARY INJECTION

AUTHOR	CONDITION OF LUNGS
1. Dogge, A. C.[13]	No pulmonary abnormalities
2. Kortright, quoted by Halstead, T. H.[15]	No pulmonary abnormalities
3. Gillette, H. F.[16]	Lungs appeared larger than normal
4. Gillette, H. F.[17]	Thymus enlarged
5. London Letter, J. A. M. A.[20]	Lungs and cavities of heart indicated sudden and extreme spasm
6. Lamson, R. W.[1]	No pulmonary abnormalities. Thymus enlarged
7. Lamson, R. W.[1]	Lungs voluminous and contained a great deal of frothy fluid
8. Gurd and Roberts[19]	Lungs voluminous and downy
9. Boughton, T. H.[2]	Lungs enormously distended and dry on section
10. McCallum, A. D.[21]	No pulmonary abnormalities noted. Right ventricle filled with fluid blood

B, FOLLOWING REINJECTION

1. Koch, W.[18]	Emphysema of lungs
2. Mendeloff, M. L.[5]	No pulmonary abnormalities noted. Thymus and spleen enlarged
3. Waugh, G. H.[12]	Congestion of lungs
4. Dean, H. R.[14]	No pulmonary abnormalities
5. Sheppe, W. M. (Herewith reported)	Lungs were markedly emphysematous and did not collapse when thoracic cavity was opened.

C, FOLLOWING REINJECTION OF POLLEN EXTRACT

1. Lamson, R. W.[4]	Lungs were distended and did not collapse when thoracic cavity was opened

The total number of necropsy reports found in the literature is fifteen. To these a sixteenth is added. Ten postmortems were performed on patients dying from a primary injection; six followed reinjection.* In ten cases an allergic history was obtained; in six it was not.

In nine instances (56 per cent) significant lung findings were demonstrated. Five of these were in primary injection deaths and four occurred following reinjection.

The thymus was recorded as enlarged in three cases (18 per cent) while marked splanchnic dilatation and liver engorgement was observed four times (25 per cent).

*No proof can be offered that the case reported belongs in the reinjection class. It is placed in this group on presumptive evidence.

DISCUSSION OF MECHANISM INVOLVED

Lamson[1, 4] and Tuft[10] have discussed very admirably the clinical characteristics and predisposing factors involved in the production of serum reactions in general and particularly in fatal reactions. Tuft classifies such reactions as (a) normal, (b) accelerated, and (c) immediate. The reader is referred to these excellent treatises for a broad conception of the subject. We will confine ourselves to a further consideration of the autopsy material provided by sixteen "immediate reactions."

There is a definite and proper hesitancy on the part of all immunologists to apply the term "anaphylaxis" to any reaction complex occurring in man. It is well known that there is to date no indisputable evidence to indicate that the clinical conditions, asthma, hay fever, or urticaria, are identical with experimental anaphylaxis (guinea pig, horse serum, bronchospasm syndrome). Similarly previous authors have decried the use of the term "fatal anaphylaxis" as applied to instances of immediate fatal reaction in man. The objections to the use of the term are as follows:

a. There is no actual experimental evidence to prove that a state of anaphylaxis may be induced in man.

b. It is not desirable to "pigeon hole" a little understood condition under the name of a definite immunologic concept. Further investigation is delayed and erroneous treatment may result.[4]

c. Such reactions in man may be produced by nonspecific factors, such as excitement, cold air, hot water, drugs, and other nonprotein excitants.

d. Failure of desensitization by the Besredka method to protect against severe reactions.

All of the above statements are cogent and worthy of full consideration. On the other hand, certain observations tend to indicate that sudden death in man, when actually produced by the injection of foreign protein, may be truly anaphylactic in character. Zinsser[11] has defined anaphylaxis as "an antigen-antibody reaction in which the inciting substance is unquestionably an antigen." In other words, anaphylaxis implies a fundamental difference between the reaction capacity of the sensitized and the normal animal. There seems no good reason to assume that this fundamental "difference in reaction capacity" cannot be developed in man as well as in lower animals. It is true that all individuals do not present the typical reaction produced by injection and reinjection of a guinea pig at the proper intervals, neither do rabbits nor dogs. Flexner and later Richet were fortunate in performing their early experiments on animals so nearly uniformly sensitizable to horse serum. Yet we can hardly state that the phenomenon of anaphylaxis is confined entirely to guinea pigs.

Further elucidation of this complex subject must come from an intensive study of the immunologic reactions in "normal" serum sickness in man. In immediate fatal reactions there is obviously no opportunity for skin tests, the demonstration of anaphylactic antibodies, or the carrying out of "passive transfer" studies. From the clinical standpoint several isolated observations are of theoretical interest. It is recognized that individuals developing serum sickness after primary injection are more apt to develop severe or even fatal reactions on reinjection.[10] Is not this evidence of an increased reaction capac-

ity of these individuals? Many authors state that in the fatal cases death was definitely respiratory in character, the heart continuing to beat long after respiration had ceased. Cooke[8] describes a death following skin testing (Le Page's glue) as follows:

"Cough and dyspnea were marked for a minute—he was deeply cyanotic and respiration ceased, though respiratory efforts continued for a minute longer. Artificial respiration was attempted but *no air could be made to enter or leave the chest.* The heart continued to beat for a minute after respiration ceased." No autopsy was obtained. It seems probable that if this and other cases had come to autopsy, evidences of bronchospasm might have been demonstrated.

The failure of methods of desensitization as ordinarily carried out cannot be offered as evidence of anything. What allergist, in attempting to desensitize his patient to feather protein for example, would expect to get results by administering four or five rapidly increasing doses within from six to twelve hours or even less time? The entire procedure is illogical and theoretically as well as practically doomed to failure.

It may be said that deaths following primary injection lack the antigenic factor of the anaphylactic triad. It is fairly well established, however, that a specific antigen may sensitize the individual either in utero or at any period of life.

Finally let us consider the evidence as presented by the autopsy reports. As has been stated, it is difficult and impractical to carry out actual immunologic studies on fatal cases. Therefore, we must constantly turn to the postmortem table. It is possible by a careful study of the anatomical changes, that we may be able to go backward step by step and establish the true pattern of events leading to death.

It seems very unlikely that the finding of "voluminous," "emphysematous," or "markedly distended lungs" in nine out of sixteen autopsies is a matter of chance. If the reports may be depended upon, the appearance of the thoracic cavities in the nine cases was certainly very unusual. In the reported case the lungs were strikingly similar in appearance to the lungs of a guinea pig dead of induced anaphylaxis. It seems reasonable to assume that the marked distension of the alveoli, failure of the lung to collapse, and increased buoyancy must indeed be due to bronchospasm.

It is quite impossible to draw conclusions of statistical value from a number of autopsy reports of foreign protein fatalities now available in the literature. We believe, however, that in at least nine such cases coming to autopsy, death was due to bronchospasm. Moreover it seems likely that this bronchospasm was induced by the injection of a lethal dose of foreign protein in an individual with an altered reaction capacity produced by a previous sensitization with the homologous protein. This sensitization may have been accidentally acquired or may have been established by previous injection. So far as these nine cases are concerned it seems that the requirements of Zinsser's definition of true anaphylaxis are fulfilled. They strongly suggest that at least in certain instances death following the injection of foreign protein may be a true anaphylactic phenomenon.

No explanation can be given for the lack of pulmonary findings in the remaining seven reports. In three of these cases the thymus was said to be enlarged. It is quite possible that the injection of foreign protein into individuals of a thymicolymphatic constitution may initiate an unknown series of events leading to death. Death occurs not infrequently in such individuals following inhalation anesthesia. The introduction of foreign protein is certainly capable of producing far more fundamental a change in the humoral and cellular physiology.

Gurd and Roberts[10] point out that the evidences of anaphylaxis in dogs are very different from the classical guinea pig syndrome. When the condition is induced experimentally in canines, the main response is evidenced by a sudden and marked capillary dilatation. This is noted especially in the liver and spleen. The entire splanchnic area may be involved. In fact, the capillaries of the kidneys and lungs may also be engorged. This is accompanied by a marked drop in blood pressure, cardiac failure, and death. It has also been noted that the visceral capillaries become engorged with leucocytes as well as red blood cells. This no doubt accounts for the leucopenia frequently noted when counts are made during the period of shock. The cases of Kortright (quoted by Halstead[13]), Dean,[11] and of Waugh[12] seem also to belong in this class. These authors (Gurd and Roberts) suggest that in man the reaction to foreign protein may follow the "dog" type (capillary dilatation and fall in blood pressure) or may conform to the "guinea pig" pattern (bronchospasm, respiratory failure). It is likely that the dog type of reaction is more compatible with life in human beings, and fortunately this may be the commonest form of reaction in man.

The lethal "shock" may act on the reticuloendothelial structures or through the autonomic nervous system.

It is not illogical to assume, therefore, that the mechanism and cause of death may vary in different cases. For this reason the blanket term "fatal anaphylaxis" is acceptable. For the same reason it is imperative that an autopsy be secured in all cases, first, to establish that death was actually due to a foreign protein reaction and not to some occult cause, and second, to demonstrate when possible the type of mechanism involved. The accumulation of pathologic material may lead to the recognition of at least three different types of fatal reaction; namely, the "bronchospastic," the "thymic," and the "splanchnic."

I would suggest that all cases lacking postmortem findings be grouped as unclassified sudden death and excluded from the literature of fatal serum reactions.

SUMMARY

1. An additional case of sudden death following the injection of foreign protein is reported with complete autopsy findings.

2. It is offered as an example of true fatal anaphylaxis of the bronchospastic type in man.

3. Fifteen other cases coming to autopsy are analyzed.

4. Evidence is offered to show that thirteen of these cases were probably examples of true anaphylaxis in man. In each of the thirteen instances death occurred either from bronchospasm or splanchnic dilatation. The theory

is advanced that an entirely different mechanism (possibly thymicolymphatic) is responsible for postinjection deaths not exhibiting bronchospasm or splanchnic dilatation at autopsy.

5. It is urged that all serum injection fatalities be subjected to autopsy for the following reasons:

a. Elimination of other and unsuspected causes of death.

b. Recording of careful observations relative to the condition of the pulmonary, thymicolymphatic, and splanchnic systems.

c. Accurate classification of type of reaction based upon anatomic findings.

The case report is used with the permission of the Department of Bacteriology and Pathology of the University of Virginia. We are indebted to Dr. Robert Graham of the same department for the preparation of the autopsy protocol.

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LABORATORY METHODS

RINGWORM OF THE FEET—VALUE OF MICROSCOPIC EXAMINATIONS*

By Harrison S. Paynter, B.S., and Cleveland White, M.D., Chicago, Ill.

RINGWORM of the feet has become a very common disease; the clinical course varies from interdigital erosions and scaling of little moment in many cases to swollen painful feet in others. In the latter case patients are sometimes disabled for weeks. Most cases are diagnosable upon clinical findings alone.

The rôle of the laboratory in the confirmation of the diagnosis of superficial fungus infections has been assayed by numerous writers; a number feel that fungi should be found before making an absolute diagnosis. In border line cases, unquestionably fungi should be diligently searched for.

The success in finding fungi by the well-known method of digestion in potassium hydroxide (so-called K O H preparation) has been reported in various percentages.

The percentages vary from thirty upward by various writers. Consequently it was decided to examine a number of cases of clinical ringworm of the toes to ascertain by this simple microscopic method the percentage of fungus cases. Sixty-five consecutive patients (all young men), who had had no previous systematic treatment, were examined. These men were being given general medical examinations and were not seeking medical treatment.

All presented interdigital erosions of the toes in one or more interspaces. The scrapings were taken from the overhanging borders and placed in 10 per cent potassium hydroxide. Usually several drops of the alkali were placed on the scrapings which had been grouped close together on an ordinary microscope slide. Upon this combination a small cover slip was laid. The low power of the microscope was first used; the high power was then used to confirm the presence of fungi. Preparations were examined at frequent intervals for at least twenty-four hours. In the cases where fungi were not recovered, scrapings were taken repeatedly (2 to 4 times).

The findings are recorded in the following table:

Hyphae	40	62 per cent
Mosaic	15	23 per cent
Yeast (budding)	8	12 per cent
Negative	2	3 per cent
Total cases	65	100 per cent

*From the Department of Dermatology, Northwestern University Medical School.

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Hyphae are the well-known thread-like filaments which are easily recognized. Mosaic forms are described by Weidman[1] as hyphae which follow the intercellular clefts, surrounding the various sized polyhedral spaces, giving a pavement effect.

There has been some dispute as to the pathogenicity of this type of fungus or as to whether it is really a fungus at all. Observations in numerous cases by one of us (C. W.) which will be published seem to amply prove that such forms are fungi and are pathogenic. In this respect we are thoroughly in accord with Weidman.[2] Greenwood[3] has concurred in this opinion and has demonstrated proof by several photographs. Yeast was considered so only if budding were found; its exact rôle may be considered questionable although clinical,[4] laboratory and experimental[5] observations apparently justify its pathogenicity in certain types of superficial dermatoses. That bacteria can cause superficial cutaneous infections simulating ringworm is a strong probability, judging by research at the Northwestern University Medical School now being carried on; the negative fungus cases may have been caused by bacteria.

Counting hyphae and mosaic forms together, fungi were then found in 85 per cent of the cases. If yeast, too, were included the percentage becomes much higher, 97 per cent. At any rate, by reasonably careful and repeated microscopic examinations, fungi may be found in at least 85 per cent of cases of clinical ringworm of the feet.

(Thanks are due to Miss Bertha Culka for assistance in making the K O H preparations.)

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FURTHER EXPERIENCES WITH THE SIMPLIFIED TECHNIC FOR HEPATECTOMY*†

By Samuel Soskin, M.D., M.A., Ph.D., Toronto, Canada

COMPLETE excision of the liver, in such animals as the dog, cat, rabbit, and monkey, is occasionally necessary for various physiologic investigations. Until Mann (1921) developed his method for hepatectomy, the usual procedure consisted in tying the hepatic artery and connecting, in some manner, the portal vein to the systemic circulation. Pavy (1899) disposed of the portal circulation by complete evisceration of the animal, then removed about 90 per cent of the liver piecemeal, by individual ligation of the hepatic veins as they enter the vena cava. This procedure removed enough liver to result in marked hypoglycemia of the animal. More recently, Bouckaert (1929) reported a method in which the portal and left renal veins are connected by a cannula, and the lobes of the liver ligated and excised one by one.

The well-known researches of Mann and his school have clearly shown that it requires but little liver tissue for the efficient performance of some hepatic functions, which cannot be carried on after complete extirpation of the liver. This fact has led to erroneous conclusions regarding metabolic functions in supposedly hepatectomized animals. The de-aminization of amino acids is a case in point (Mathews, 1921). The complete excision of the liver without other disturbance to the animal, accomplished by Mann, marked the beginning of the accumulation of definite facts concerning the physiology of this viscus.

Mann's method for hepatectomy requires two preliminary operations before the liver can be removed at a third operation. In the first operation a reverse Eck fistula is performed so that the blood from the inferior vena cava is diverted into the portal vein. The resulting increased pressure in the portal system is followed by the development of an extensive collateral circulation connecting the portal and systemic venous radicles. At a second operation, three or more weeks after the first, the portal vein is tied, completely if possible, incomplete ligation being practiced when marked cyanosis of the intestines develops. At a subsequent operation, which may be done at any time, the liver is removed in one piece.

A consideration of this technic makes it apparent that the principle of Mann's three-stage method is the development of a collateral circulation for the portal vein and vena cava, by increasing the venous pressure in these vessels. After this condition has persisted for some time, both trunks may be completely ligated and the blood follows the newly formed vessels to the

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heart. Accordingly Markowitz and Soskin (1927) proposed a simplified technic for removing the liver, based upon the principles utilized by Mann. The method, in brief, consists in incomplete ligation of the portal vein and inferior vena cava, so as to establish a considerable back pressure in these vessels. At a subsequent operation, these vessels are completely tied and the liver removed.

The simplification effected by the technic of Markowitz and Soskin is apparent. There can be no question that in Dr. Mann's hands, the excision of the liver by his three-stage operation is a highly feasible procedure. But to the physiologist who has not specialized in this type of technic, his methods are difficult to apply. The three-stage technic, moreover, has the disadvantage that it is not applicable to smaller animals like cats and rabbits, in which Eck fistulas cannot satisfactorily be produced. Heretofore, the investigator who has wished to study the hepatectomized animal has resorted to various approximations. Unfortunately, these approximate hepatectomies must be condemned, not only because functioning liver tissue remains, but also because the animal may contain a considerable amount of necrotic liver tissue, the toxicity of which, as Mann has shown, is extraordinary. Since a simple technic for complete hepatectomy is now available, the use of these approximate hepatectomies is unnecessary.

During the past three years, the author has had the opportunity of making an extensive trial of Markowitz and Soskin's simplified technic for hepatectomy. In his hands, the operation has been simple and extremely feasible. Although no attempt was made to determine the maximum survival period of such a preparation, there is every reason to believe that, under suitable conditions, the preparation will survive as long as those prepared by Mann's three-stage operation. Following the original report of this technic, other laboratories have satisfactorily employed our procedure to remove the liver from dogs and other animals. This technic makes it possible to remove the liver from a rabbit, as completely as from the dog. As it stands, however, the first operation is followed by a tremendous number of vascular adhesions in the rabbit, which makes the final operation unnecessarily difficult. Drury (1929), accordingly, has made an ingenious modification of the method, whereby these adhesions are avoided. He has reported a survival period of forty hours for the hepatectomized rabbit. Other laboratories are employing our technic for dehepatizing cats and monkeys (personal communications), with satisfactory results. The method has also been successfully employed by Markowitz, Mann and Bollman (1929).

Markowitz and Soskin's simplified technic for hepatectomy, briefly outlined in a previous publication, is described in detail below.

TECHNIC

Under ether anesthesia, and with aseptic precautions, the abdomen is opened through a midline or right paramedian incision. The intestines, suitably protected with warm moist towels, are strongly retracted to the left. The vena cava, proximal to the lumboadrenal vein, is exposed and a hiatus created about it with the thumb and forefinger. An aneurysm hook threaded

with *stout* linen is passed through the hiatus, and the vena cava is occluded so that ⅔ to ⅘ of its lumen is obliterated. With practice, it is possible to pass a suitably threaded aneurysm hook about the vena cava, without previous blunt dissection. Following this procedure fewer adhesions are apt to occur. The degree to which the ligature is tightened depends on the back pressure produced. This part of the technic requires judgment. When the ligature is too tight, the vena cava below it rapidly develops a tension which, on palpation, is comparable to that of the normal eyeball. When this occurs the ligature should be slightly loosened until palpation of the obstructed vein yields the sensation of elastic resiliency, which the operator soon learns to recognize. It is important that the ligature be not too loose, since obviously a collateral circulation will not then develop. A similar procedure is repeated on the portal vein. An aneurysm hook, similarly threaded with stout linen, is passed around the portal vein proximal to its last tributary. The degree to which the ligature is tightened is about the same as for the vena cava, and is conditioned by the appearance of the intestines. These should be congested and mildly cyanotic in color, but not definitely blue. The abdomen is closed in three layers.

At a subsequent operation, which is best done four or more weeks later, the liver is removed as follows:

The abdomen is opened, and omental adhesions are carefully cut between ligatures until the structures in the lesser omentum are exposed. It may be necessary to separate, with the finger, some filmy adhesions of the liver to surrounding structures. These rarely bleed. The structures in the lesser omentum are tied en masse with stout linen, then cut between ligatures, care being taken not to occlude the last tributary of the portal vein. The vena cava, even if already completely occluded by the inflammatory reaction about the ligature, is tied and cut at the previous operative site. To dispose of the diaphragmatic portion of the vena cava, the hand is inserted between the liver and the diaphragm. With the thumb and forefinger, the liver is freed from a great part of its attachment to the vena cava and coronary ligament. The liver is then retracted caudalward and a Hoffmann burette clamp with a swivel side-arm is placed around the exposed liver pedicle and securely tightened. If desired, a ligature may be placed proximal to the clamp; or a ligature can be secured around the vena cava by transfixing it through the adjacent portions of the diaphragm, thus obviating the use of a burette clamp. The liver is now excised. Since many hepatectomized dogs show a certain amount of bloody fluid in the peritoneal cavity at autopsy, a careful search of the intestines and diaphragm for bleeding points is always advisable. These should be carefully ligatured. When the bleeding is general, dry gauze sponges may be left in the abdomen over the oozing areas, to facilitate hemostasis. The abdomen is closed in three layers.

Rigid asepsis is as advisable for the second operation as for the first. Especially is this the case in the occasional animal in which collateral circulation has not developed sufficiently to make a satisfactory hepatectomy possible. When this is found to be the case, the portal vein and vena cava may be completely ligated and the abdominal incision closed. This operation will

be well borne, when the added burden of hepatectomy might prove fatal. At a subsequent operation, two or more days later, the liver can be satisfactorily removed.

The difficulty of hepatectomy, by either Mann's or Markowitz and Soskin's method, depends largely upon the number of adhesions encountered at the final operation. Some dogs develop adhesions in the abdominal cavity very readily, and any technic for forcing a collateral circulation will be followed by the appearance of massive vascular adhesions, which must be carefully ligated. Some animals, on the other hand, are so constituted that even extensive intraperitoneal trauma is not followed by severe adhesions. Such animals are ideal for hepatectomy preparations, the absence of adhesions rendering the removal of the liver not much more difficult than a nephrectomy.

A one-stage method for hepatectomy has recently been described. (Firor and Stinson, 1929). This method depends upon the use of a Pyrex glass cannula to connect the portal vein and infrahepatic portion of the inferior vena cava to the suprahepatic portion of the vena cava. Without going into the question of the cause of death in such preparations, it must be pointed out that each of the stages in the technic of Markowitz and Soskin is a relatively simple procedure, and after the final operation one is left with a hepatectomized animal, which is otherwise apparently normal. This latter is not the case for any other method except Mann's three-stage operation. The technic described by us is much simpler than any of the approximate hepatectomies described in the literature.

SUMMARY

The simplified technic for hepatectomy of Markowitz and Soskin is described in some detail, and its advantages briefly discussed.

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THE SCHILLING INDEX IN APPENDICITIS*

By Raymond H. Goodale, M.D., and M. Evelyn Manning, Ph.G.,
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IT IS the object of this paper to compare the Schilling index with the microscopic pathologic findings in a series of forty operated appendices. A leucocyte count and usually a differential count constitute the routine blood examination in cases of suspected appendicitis in the modern hospital. Schilling's modification of the Arneth index gives us an additional aid without appreciably increasing the length of time or labor involved.

The leucocyte counts and the blood smears were made two to fifteen hours before operation. The relation to meals was not considered since Rosenfeld[1] and others claim that it does not affect the differential count. The smears were made on slides and were stained with Wright's stain at the time when the leucocyte counts were done. The procedure followed in doing the differential counts was that described by Schilling[2] in his book, *The Blood Picture*. We used Schilling's terminology and normal differential percentages, namely: basophiles 0.5 per cent, eosinophiles 2 to 4 per cent, myelocytes 0 per cent, juvenile forms 0 per cent, stab forms 3 to 5 per cent, segmented forms 58 to 66 per cent, lymphocytes 21 to 25 per cent, large mononuclears 4 to 8 per cent. These normals checked with a series of 25 normal smears done in this laboratory as controls. Schilling describes the forms of the neutrophilic granulocytes used in his differential as follows:

"Myelocytes—the nucleus is round, oval or kidney shaped; relatively large, vesicular, coarsely granular, usually has a nucleolus, and takes a pale stain . . . . they are normally found only in the bone marrow.

"Juvenile forms (jugendliche)—normally many are found in the bone marrow, rarely in the peripheral blood. The nucleus is sausage and bean-shaped. Its consisteney is vesicular, and does not stain intensely . . . . these cells are usually slightly larger than the mature cells. . . . .

"Staff or stab forms (stabkernige)—the nucleus is T, U or V shaped. The cytoplasm is fully mature. These cells are differentiated from adult forms and juvenile forms by a small band-like, often bizarre, twisted and always hyperchromatic (dark structureless) nuclear form. . . . .

"Segmented forms—the nucleus consists of 2 to 5 unequal segments. The latter are united by fine threads."

These forms are illustrated in Fig. 1.

An increase in the percentage of one or more of the young forms in the blood smear is called a "shift to the left" and is indicative of pathology. This shift to the left may take place even though there is no increase in the leucocyte count.

*From the Pathological Laboratory, City Hospital.
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One hundred cells were counted in each of the smears and in half of them 200 cells were counted. A mechanical stage was used in order to avoid counting any part of the field twice. It will be noted that only the neutrophilic granulocytes are listed in the charts since they are the only ones which pertain to this work.

The appendices were placed in 10 per cent formalin immediately after removal, dehydrated in alcohol, sectioned in paraffin and stained with hematoxylin and eosin. All appendices were examined and described grossly. Fecal matter with or without pus was found in the lumen of all specimens except those in which the lumen was obliterated. In three, Oxyuris vermicularis was found. A larger series might be presented, but it is felt that these are fairly illustrative. Since we started the Schilling index, we have not encountered any of the less common lesions of the appendix such as tuberculosis and malignancy. No incidental appendices were included in this series.

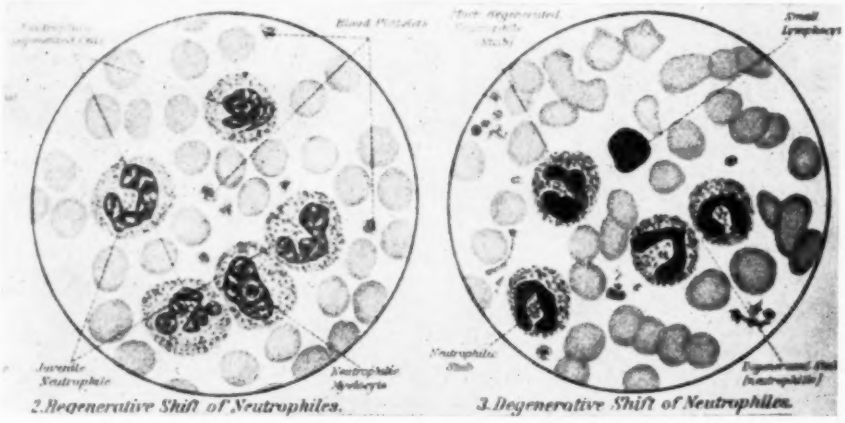

Fig. 1.—Various forms of neutrophiles. (From Schilling.)

DISCUSSION

The various forms of neutrophiles, the white counts, the pathologic findings and the age and sex of the patients are listed in the charts. The age is given because children have a high white count at times, with very little pathology. For convenience the ten acute appendices are listed in one chart, and those with more chronic pathology are listed in a second chart. The diagnoses are not given except in the above general way because we feel that a given diagnosis, with the possible exception of acute appendicitis, does not convey the same picture to everyone.

The clinical features of the cases can be discussed only in brief. All of the patients presented an appendix syndrome which seemed to warrant operation. The abdominal pain was not due to an acute inflammatory process except in the ten cases in Table I. In the remaining thirty the pain must have been due to: (1) muscular contraction around the feces in the lumen, (2) stretching of the mucosa due to hyperplasia of the reticulum cells of the lymph follicles, or (3) some cause outside of the appendix.

The question now presents itself: Can we more accurately visualize the pathologic process in the appendix with the aid of the Schilling index? This series of cases was examined in retrospect, but recently the Schilling index in conjunction with other findings has helped us to prophecy what the appendix would show in a few cases which we have had the opportunity of observing before operation.

In those cases in Table I where the appendices showed a general polymorphonuclear infiltration with or without pus in the lumen there is a definite shift to the left, i.e., an increase in the juvenile or stab forms or both. This indicates a stimulation of the bone marrow which sends out young neutrophiles after the storage depots have been exhausted. Taking 6000 to

TABLE I

SCHILLING INDEX IN ACUTE APPENDICES

CASE NO.	AGE	SEX	WHITE BLOOD CELLS	MYELOCYTES	JUVENILE FORMS	STAB FORMS	SEGMENTED FORMS	PATHOLOGIC FINDINGS
1	39	M	16,500	0	1	13	70	Polymorphonuclears in all layers of wall
2	11	M	14,100	0	0	14	73	Polymorphonuclears in all layers of wall
3	47	M	20,000	1	5	15	75	Polymorphonuclears in all layers of wall and in lumen
4	48	M	18,400	0	1	35	46	Polymorphonuclears in all layers of wall and in lumen
5	52	F	10,500	0	3	10	64	Polymorphonuclears in all layers of wall and in lumen
6	27	M	15,700	0	0	17	58	Fibrin on surface containing polymorphonuclears and lymphocytes
7	40	F	22,400	0	5	30	54	Polymorphonuclears in all layers, in lumen and on surface
8	43	F	15,000	0	3	23	71	Polymorphonuclears in all layers and in lumen
9	13	F	29,800	0	0	11	78	Polymorphonuclears in muscularis and on surface
10	7	M	27,400	0	0	18	70	Polymorphonuclears in muscularis and serosa

8000 white cells per c.mm. as normal, all of these acute cases had a leucocytosis. But the white count is not as sensitive as the Schilling index as seen in Case 5 where the count is only 10,500. Yet there is a definite shift to the left (3 juvenile, 10 stab, 64 adult forms), indicating an acute infection. On the other hand, white counts higher than this will be noted in Table II with no increase in the young forms and no acute pathology in the appendices.

The increase in total percentage of neutrophiles as determined in the ordinary differential has been used as evidence of an acute infection. Such an increase will be found in both charts by adding together the young and adult forms. In Table II those showing an increase in percentage of neutrophiles do not have acute pathology in the appendices. The differentials

TABLE II

SCHILLING INDEX IN NONACUTE APPENDICES

CASE NO.	AGE	SEX	WHITE BLOOD CELLS	MYELOCYTES	JUVENILE FORMS	STAB FORMS	SEGMENTED FORMS	PATHOLOGIC FINDINGS
1	13	M	7,200	0	0	1	60	No reaction in wall
2	19	M	8,600	0	0	3	49	Few round cells in serosa
3	29	M	12,400	0	0	2	62	Hyperplasia of reticulum cells of lymph follicles
4	17	M	9,050	0	0	5	54	No reaction in wall
5	41	M	13,000	0	0	5	80	Hyperplasia of reticulum cells of lymph follicles
6	14	F	7,500	0	0	1	59	Few round cells in serosa
7	47	M	13,100	0	0	5	64	Mucosa and submucosa replaced by connective tissue
8	12	F	17,800	0	3	8	74	Hyperplasia of reticulum cells of lymph follicles, Oxyuris in lumen
9	17	F	7,200	0	0	7	58	Hyperplasia of reticulum cells of lymph follicles
10	9	M	6,700	0	0	4	70	Hyperplasia of reticulum cells of lymph follicles, Oxyuris in lumen
11	17	F	11,900	0	0	2	47	Hyperplasia of reticulum cells of lymph follicles
12	10	F	6,800	0	0	6	41	Hyperplasia of reticulum cells of lymph follicles
13	57	M	11,900	0	0	7	84	No reaction in wall
14	24	F	14,200	0	0	2	80	Lumen filled with connective tissue
15	34	M	8,900	0	0	2	72	Few foci of lymphocytes in muscularis
16	12	F	21,400	0	0	7	75	No reaction in wall, Oxyuris in lumen
17	19	M	11,600	0	0	3	31	Slight hyperplasia of reticulum cells of lymph follicles
18	18	F	9,000	0	0	5	59	Hyperplasia of reticulum cells of lymph follicles
19	12	M	8,000	0	0	3	84	Few round cells in serosa
20	17	F	8,600	0	0	3	74	Round cells on surface
21	15	F	9,800	0	0	3	63	Hyperplasia of reticulum cells of lymph follicles
22	22	F	7,400	0	0	1	78	Hyperplasia of reticulum cells of lymph follicles
23	12	M	9,700	0	0	5	33	Hyperplasia of reticulum cells of lymph follicles
24	18	F	24,400	0	0	1	87	Fat cells in submucosa. Few lymphocytes in muscularis
25	30	F	8,100	0	0	7	72	Hyperplasia of reticulum cells of lymph follicles
26	40	M	12,400	0	0	2	66	Lumen obliterated. Fat in submucosa
27	43	M	16,600	0	1	11	61	Hyperplasia of reticulum cells of lymph follicles
28	20	M	16,200	0	0	9	71	Few lymphocytes in muscularis
29	25	M	13,500	0	0	5	66	Few eosinophiles in muscularis and serosa
30	23	F	9,000	0	0	7	63	Hyperplasia of reticulum cells of lymph follicles

in Table II show little or no shift to the left and therefore reflect the pathology of the appendices more accurately.

In Table II it will be noted that the stab forms vary from 1 to 9 per cent (normal 3 to 5 per cent), and the white counts vary from 6,700 to 16,200. The pathology is of mild chronic type except in three cases where there was no reaction in the wall. In Case 28 the appendix showed a few lymphocytes in the muscularis with a white count of 16,200. The slight shift of 9 per cent stab forms is more consistent with this mild pathology than is the white count.

Two cases, 8 and 27, with white counts of 17,800 and 16,000 respectively, both with an increase in young forms and only hyperplasia of the lymph follicles would indicate that one should look elsewhere in the body for the acute process.

Reznikoff[3] cited a case in which the Schilling differential was very helpful as confirmatory evidence in differentiating between an acute appendix and a gastrointestinal upset. The patient had a white count of 5,400 with 6 per cent stab forms and 53 per cent adult forms. After an enema all symptoms cleared up.

SUMMARY

The Schilling index, white count and microscopic pathology are presented in each of a series of forty operated appendices, ten of which were found to have an acute inflammatory process. There is a definite shift to the left or an increase in young forms of the neutrophiles in the acute appendices. The remaining appendices showing no acute pathology have little or no increase in young forms despite an increase in several of the white counts. The white counts and total percentage of neutrophiles do not always run parallel with the infection in the appendix.

In view of these findings we believe that the Schilling index in addition to the white count renders a more accurate picture of the pathology in the appendix than the white count with the ordinary differential.

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A METHOD FOR THE GERMICIDAL ASSAY OF SOAPS*

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SINCE one of us first discussed the subject of soaps as germicidal agents,[1] much has been written and many new facts established. In most cases every advance has suggested new problems and shown that unrecognized factors may be responsible for the recorded observations. Among these to be noted as of primary importance are the glyceride from which the soap is prepared,[2] and the organism against which it is tested.[3] In most instances the subject has been treated without immediate reference to the practical side of the question, so that high values have been recorded for soaps of unusual glycerides, alpha brom soaps for example,[4] and especially when tested against certain less common species of bacteria, certain strains of streptococcus,[5] B. dysentery, meningococcus, B. paratyphosus.[6] There are several phases of the subject more or less related and yet of sufficient individual interest as to suggest separate consideration; first, the practical value of the ordinary soaps which one would purchase for cleansing purposes,[7, 8] second, the extraordinary effectiveness of soaps of unusual composition, soaps of lauric and linolenic acids,[9, 2] third, the selective features by which certain species of microorganisms respond differently to soaps from different glycerides, for example, Treponema pallidum,[3] diplococcus pneumoniae,[10] and Vincent's bacillus,[10] fourth, the effect of these soaps on associated substances of known germicidal efficiency, cresylic acid,[1, 11] phenol,[12] mercuric iodide.[13]

This paper is devoted to a consideration of the practical value of substances in common use and especially to methods of evaluation. This is of the greatest importance because the confusion that exists today as to the real efficiency of soaps and other substances commonly used for cleansing and disinfecting the skin is directly due to the lack of a clear-cut practical method as distinguished from the purely laboratory methods of measuring values.

A senior pharmacy student's paper[14] of eleven years ago still does service in attempting to discredit one of the most generally effective disinfectants known. A later research by Norton,[6] undertaken in an honest endeavor to throw light on the subject, produced negative results only. Both investigators reached wrong conclusions because of an unfortunate choice of method in applying the tests.

In choosing a method for germicidal assay, there are two features to consider: first, to select a procedure readily adapted to laboratory comparisons, second, if possible, to demonstrate the efficiency under practical conditions. The first should be regarded as a laboratory control method for maintaining uniformity in an established product. The methods commonly used for assay of the coal tar disinfectants are in general equally applicable to other classes of disinfectants. They require only some slight modifications in order that

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the results obtained may be used with confidence as relative values for laboratory control and comparison with similar preparations.

It is quite appropriate, therefore, to select for germicidal soaps and many other substances, the Hygienic Laboratory Method.[17] This is the method which has been used by us ever since its inception for the routine assay of our germicidal soap containing as its active agent mercuric iodide* in the form of a soluble double salt with potassium iodide.

On the other hand a procedure based on practical use is the only way to assure oneself of the value of a substance subjected to such a test. It is imperative that any assertion of efficiency should be supported by definite proof in the form of scientific data. In selecting such a method for demonstrating the effectiveness of substances recommended for disinfecting the skin, one must not be misled into attempting the impossible nor should one impose conditions unnecessarily severe. On the other hand, merely to demonstrate that a substance is germicidal is not sufficient.

Norton[7] selected the logical place to bring about a sterile field, the hands, but failed to take into account the fact, acknowledged by physician, and bacteriologist alike, that it is impossible to render the skin of the hands sterile by any treatment which will leave the skin intact if frequent applications are necessary. Hence the donning of surgeons sterilized rubber gloves before performing any operation where contamination would be a calamity.

Attempts to prove the value of a disinfectant on the smooth surface of these rubber gloves disclosed the fact that the detergent action of ordinary soap alone, used with a reasonable degree of diligence and care, will remove the contaminating organisms quite completely.

The skin of animals has been used and is most promising but the results obtained by Reddish and Drake[15] and Simmons[16] are so contradictory as to indicate that the procedure is in need of careful revision.

The animal finally selected in our work was the guinea pig. The procedure† is simple and lends itself to the examination of a great variety of substances. For this research, however, only a limited number of substances was examined but sufficient, it is thought, amply to demonstrate the adaptability of this method to measure the efficiency of a substance recommended for disinfection of the skin.

The skin over the abdomen of the guinea pig was lathered with a germicidal soap, shaved, rinsed off thoroughly with sterile water and a culture taken. A second lathering for one-half minute was done with the same soap, then the skin washed and cultured. Very rarely was the first culture sterile and equally rarely was the second contaminated. Then a twenty-four

*One would expect all soaps containing 1 per cent of mercuric iodide to be equivalent in their germicidal values. Tests of nine different commercial samples, however, show wide variation in bactericidal activity, a range from 8 to 65 in phenol coefficients.

†A method by Raizise, et al., (18) published between the presentation and the publication of this paper, is very similar in technic to the method here described. The outstanding differences are: the use of a depilatory instead of the razor for removing the hair, the procedure in applying the disinfectant, and the time of contact of the disinfectant with the skin.

The results where comparison is possible are quite similar to ours.

There are certain features which seem to limit the practicability of the method, chiefly in the fact that the germicide was applied to the skin over a long period (five minutes) and contact was permitted over much longer periods (five to sixty minutes).

The method of applying the disinfectant too (by means of gauze laid on for five minutes) is so far removed from the practical that one would be inclined to discount the results unless verified by a more logical procedure.

hour, fluid-medium culture of Staphylococcus aureus was applied evenly over the shaved abdominal surface. It was permitted to dry five to ten minutes and a culture taken as a control.

These cultures were taken from the skin by means of sterile swabs, first dipped into a tube of sterile culture medium to moisten the cotton, then lightly rubbed over a representative portion of the surface and finally returned to the tube of medium. The long wooden applicator on which the cotton is wrapped was either broken off or held to one side to permit returning the cotton plug.

Then, keeping to the left side of the median line of the abdominal area, the shaved surface was lightly rubbed with the solution to be tested and at the end of the selected time period, a culture was removed as described above.

In order to eliminate the possibility of any inhibiting action of the disinfectant removed by the swab, the latter was twirled in a first tube and then transferred to a second tube of medium. The same procedure was followed with the right side of the pig with the one precaution that the solutions used on the two sides were selected with the intention that one would probably kill the test organism while the other would not. This was for the purpose of so controlling the test that with the exception of the solution used, the conditions were identical on both sides. In some cases the hay bacillus or other sporebearer was present so that growth showed alike from both sides.

In the earlier experiments the disinfectant was in contact for three minutes but this prolonged period was found to be not only unnecessary but an unreasonable length of time to wait for disinfection. In later tests one minute was selected as the time of application and this again was shortened to one-half minute.

The solutions tested were warmed to 40° to 45° C. partly because soap solutions, the higher concentrations, are not fluid at low temperature and partly because washing, particularly when practiced for disinfecting purposes, is almost invariably at a temperature above that of the body.

Staphylococcus aureus was selected as the test organism because it has high resistance to disinfectants, is commonly found on the skin and is a pathogenic, nonspore-bearing organism which readily grows in ordinary fluid medium.

The substances selected for testing were a solid and a fluid soap containing mercuric potassium iodide, solid and fluid soaps without added germicidal constituents, phenol, tincture iodine, alcohol, and mercurochrome. In order to avoid variable results due to the use of soaps with possibly varying contents of mercury, the experiments were confined to one brand of germicidal soap only.

The results as shown in Tables I to IV are interesting and striking.

It is shown that alcohol is uniformly ineffective in any concentration, that tincture iodine can be diluted with two volumes of water and remain effective, that phenol is effective in a 1 to 50 dilution, that toilet or washing soaps are wholly ineffective, that the soap containing 1 per cent mercuric iodide is effective in one-half minute when diluted 1-50 but fails when diluted 1-100. When the time of contact is one-half minute, there are failures with

TABLE I

GERMICIDAL ASSAY. HYGIENIC LABORATORY METHOD

	Germicidal Soap, 1 Per Cent Mercuric Iodide 5	10	15 minutes		
1-4000	–	–	–		50
5000	+	–	–		55
6000	+	+	–		60
7000	+	+	+	3	165
8000	+	+	+		55
	Phenol				
1- 80	–	–	–		
90	+	–	–		
100	+	+	–		

TABLE II

TYPICAL REPORT ON SOAP EFFICIENCY ON SKIN OF GUINEA PIG

Lather with germicidal soap, shave, wash, culture	+
A second lather, wash, culture	–
Apply Staphylococcus aureus, dry, culture	+
Left side—1 per cent solution of germicidal soap, culture	+
Right side—2 per cent solution of germicidal soap, culture	–

Soap solution kept at 45° C. and applied with swab for one-half minute. Results recorded after twenty-four to seventy-two hours.

TABLE III

SUMMARY OF SKIN TESTS OF GERMICIDAL SOAP
TEST ORGANISM, STAPH. AUREUS
TIME OF CONTACT, ONE-HALF MINUTE

Germicidal Soap (containing 1 per cent HgI_2)	
1 per cent solution	+++++++++
2 per cent solution	+–––––––+–
3 per cent solution	––––––+–
4 per cent solution	–––
5 per cent solution	–––

Plus sign (+) growth; minus sign (–) no growth. Each plus (+) or minus (–) sign in Tables III and IV is the record of a single experiment.

TABLE IV

GERMICIDAL TESTS ON SKIN OF GUINEA PIGS

Alcohol		Tr. Iodine diluted with water	
95 per cent solution	+ +	50 per cent solution	– –
70 per cent solution	+ +	33⅓ per cent solution	– – –
50 per cent solution	+ + – +	30 per cent solution	– – –
30 per cent solution	+ +	25 per cent solution	+ + +
		20 per cent solution	+ +
Liquid Soap (no germicide)		Liquid Neko (containing ¼ per cent HgI_2)	
6 per cent solution	+ + +	6 per cent solution	– + + – –
8 per cent solution	+ + +	8 per cent solution	– – – – –
12 per cent solution	+ + +	12 per cent solution	– – –
Phenol		Lenox Soap	
5 per cent solution	– –	2 per cent solution	+ + +
3 per cent solution	– – –	3 per cent solution	+ + +
2 per cent solution	– – –	4 per cent solution	+ + +
1 per cent solution	+ + +	5 per cent solution	+ + +

the 1-50 dilution of this germicidal soap but the successful results are more than 50 per cent of the experiments.

The period of time permitted to elapse between applying the contaminating organism and applying the disinfectant was limited to ten minutes or sufficient for complete drying. If, as was the practice of Reddish and Drake[14] and Simmons,[15] the time had been extended to hours, contamination with other organisms and particularly spore-bearers would intend to increase the probability of variable results without obtaining values more truly representative from the point of view of relative efficiency. From the practical point of view a good germicidal soap applied with reasonable care should have greater value because the lather is almost invariably higher in soap content than the 2 per cent solution which was found efficient in the tests.

The control demonstrates:

First, that a second lathering with an active germicidal soap after shaving is necessary and usually effective in killing or removing spore-bearers and all pathogenic organisms.

Second, that the application of the contaminating organism was fairly uniform.

Third, that the effectiveness of the disinfectant does not depend upon mechanical removal of the contaminating organisms.

Fourth, that in no instance were the results affected by the inhibiting action of the disinfectant carried over into the subculture.

CONCLUSIONS

1. The skin of guinea pigs is a satisfactory field for the testing of disinfectants commonly used on the skin.

2. A good germicidal soap can be demonstrated on a practical basis as well as by laboratory test.

3. Phenol is a much more dependable skin disinfectant than is recognized, and is effective in a safe dilution.

4. Alcohol applied before making injections or minor operations is practically without germicidal value.

5. Tincture iodine is a valuable skin disinfectant.

6. Mercuric iodide properly incorporated in a soap base in a concentration of 1-100 has been shown to be an effective agent for skin disinfection.

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A CULTURE OF B. MORGANII NO. 1 ISOLATED FROM A PARROT*

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ONE of the difficulties encountered in the frequent attempts to demonstrate the pathogenic significance of B. morganii No. 1 is the serologic specificity of the strain of these organisms, although Havens and Ridgeway[1] have shown some antigenic relationship among certain strains by means of the complement-fixation test. Morgan[2] reported very little cross agglutination, but of seventeen strains isolated in cases of summer diarrhea in children, four were agglutinated in a 1:2000 dilution, and five in a 1:200 dilution of serum prepared with one of them. Other investigators[3, 7] have failed to find any evidence of serologic relationship. During the past six years, twenty strains of organisms having the morphologic and cultural characteristics of Morgan's bacillus have been studied in the Albany laboratory, none of which was agglutinated in sera prepared with the standard strain.† Rabbits were immunized with three of the unidentified cultures, but sera thus produced agglutinated only the homologous strains. Hence, considerable interest was aroused by the isolation of a culture which was agglutinated in and absorbed the agglutinative properties from serum prepared with the standard strain.

No less interesting than its identity is the origin of this culture. It was isolated from the heart's blood, small intestine, and esophagus at autopsy of a parrot which was one of several purchased in January, 1930, for use in a laboratory study of so-called psittacosis. The bird, which was uninoculated, died five days after arrival at the laboratory. The only symptom of illness was bloody diarrhea, and the autopsy showed no abnormalities except a small abscess in the wall of the cloaca. It is of course impossible to say what part, if any,

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†The standard strain which has been used for comparison and with which the agglutinating serum was prepared was received from the Lister Institute in 1921.

Morgan's bacillus played in the death of the bird. Another parrot which was kept in the same cage died twenty-two days later; it had neither been inoculated nor shown any symptoms of illness. No abnormalities were noted at autopsy and no organisms of known pathogenic significance were isolated from the organs. Lowell[8] has reported the association of B. morganii No. 1 with fatal infections in mammals, birds and reptiles in the zoological gardens in London.

In view of the serologic reactions there can be no doubt as to the identity of the culture. However, an irregularity observed in its biochemical activities requires special consideration. Morgan's bacillus No. 1 is a motile, gram-negative bacillus which produces acid and gas in the monosaccharides, dextrose, galactose and levulose, but not in di- or polysaccharides. The strain isolated from the parrot ferments, in addition to the monosaccharides, the disaccharide saccharose. This variation seems of little import from the standpoint of identification, since it is not unusual for strains of certain organisms, for example dysentery bacilli, to vary in their fermentative reactions. It must be emphasized, however, that the fermentation of saccharose produces a reaction characteristic of B. coli in triple-sugar agar, although the gas formation is appreciably less than that of most strains of the latter. It is obvious, therefore, that such organisms may be overlooked in laboratories using triple-sugar medium for differentiating cultures isolated from specimens submitted from cases of enteric disease. Aside from its academic interest, this study serves to bring to our attention the existence of aberrant strains and the fallacy of basing identification of organisms upon an inadequate series of tests.

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A MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS WITH SPINAL FLUID*

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THE microscopic slide precipitation test for syphilis with spinal fluid described below is somewhat more sensitive than the Wassermann test with the same antigen and much simpler in detail, requiring no titrated hemolytic system of cells, amboceptor and complement. Furthermore, it requires no concentration of the globulins of the spinal fluid, it is performed with antigen emulsions that are satisfactory for use for twenty-four hours, and the results of the reaction magnified about 120 times by the microscope are read accurately with ease.

PRELIMINARY PROCEDURES WITH SPINAL FLUIDS

Spinal fluids turbid with blood, exudate or bacteria, or containing injected substances including horse serum are unsatisfactory for testing. Spinal fluids with slight turbidity or few particles are centrifuged at high speed for ten minutes and the clear fluid withdrawn or decanted. All clear and cleared spinal fluids are tested as follows:

Place the required number of test tubes $6 \times \frac{5}{8}$ inch each containing 5 c.c. of Benedicts solution (1926) in a beaker (Pyrex). Add water halfway to the top. Heat. Keep the tubes in vigorously boiling water five minutes.

Place the tubes in a rack. After making certain no copper reduction has occurred in any of the tubes, add to each tube properly numbered 0.5 c.c. of spinal fluid. Shake the tubes individually vigorously for ten seconds.

Replace the tubes in the beaker. Add water halfway to the top. Heat. Keep the tubes in vigorously boiling water five minutes.

Replace the tubes in the rack, inspecting each immediately upon removal from the beaker for precipitate indicating presence of sugar.

Spinal fluids giving a negative reaction for sugar in the above test are unsatisfactory for testing for syphilis. These are fluids that have been acted upon by bacteria either inside or outside of the body. In the former case of the various bacterial meningitides in which organisms and ferments of the exudate have acted upon the sugar,[1] the fluids may contain substances giving positive or unsatisfactory reactions in various tests for syphilis. In the latter case of bacterial contamination subsequent to withdrawal, spinal fluids from cases of syphilis show a steady loss both of sugar and of the specific reacting substance if the fluids are kept at room temperature.

On the other hand spinal fluids containing sugar when withdrawn from the body kept at low temperature (8° to 12° C.) continue to give a positive

*From the Laboratory Department of Mount Sinai Hospital.
Received for publication, July 8, 1930.

reaction for sugar with the test described above for several weeks, and syphilitic spinal fluids under these conditions show no appreciable loss of specific reacting substance for at least a week.

The clear and cleared spinal fluids which give a positive reaction in the sugar test described above are then tested as follows:

MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS WITH SPINAL FLUID

Into each of twelve chambers on six glass slides, deliver 0.05 c.c. of 1 per cent glacial acetic acid solution from a 0.2 c.c. pipette graduated in thousandths.

Into each chamber allow 0.25 c.c. of the spinal fluid to be tested to fall from a 1 c.c. pipette graduated in hundredths. (Hold the pipette directly above the acid and lastly touch the tip of the pipette at some dry portion of the chamber.)

Rotate the slides in a holder on a flat surface with moderate vigor for one minute.

Into each chamber allow one drop (about 0.0075 c.c.) of concentrated antigen emulsion to fall from a Wright pipette.

Rotate the slides in the holder on a flat surface with moderate vigor for one minute to distribute the antigen and then for four minutes more the holder gently, but rapidly (about three complex movements a second), back and forth a distance of one-fourth to one-half inch.

The results are examined at once through the microscope at a magnification of about 120 times (eyepiece 12X, objective 16 mm.) with the light cut down as for the study of urinary sediments and recorded in terms of pluses according to the degree of clumping and the size of the clumps. (For ease in reading the results, the slide is tilted on a piece of metal $\frac{1}{8}$ inch thick, 1 inch wide, and 4 inches long, placed on the stage.)

MATERIALS FOR THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS WITH SPINAL FLUID

Glassware.—Microscopic slides, 3 × 2 inches, each with two wax rings made as follows: A 3 × 2 inch microscopic slide is covered on both sides with bon ami paste. After the paste has dried (in a few minutes), it is rubbed off with a clean soft muslin cloth leaving a clean smooth surface. Upon the clean slide a steel mold $3\frac{7}{16} \times 2\frac{3}{16} \times \frac{1}{8}$ inches with two central wells $1\frac{9}{16}$ inches in diameter is placed. A metal disc $1\frac{5}{16}$ inches in diameter and $\frac{3}{16}$ of an inch thick is then placed in the center of each well. The space between them is filled with hot wax (2 parts ordinary vaseline, 1 part parowax) from a 10 c.c. glass syringe. After the mixture cools a few minutes, the mold is removed by inserting a thin blade between it and the slide. See illustration in a previous article.[2]

The pipettes used for delivering the spinal fluid are the ordinary 1 c.c. pipettes graduated in 0.01 c.c. The pipette for the antigen emulsion is a Wright pipette made from glass tubing 8 to 10 mm. in diameter with a fine tube about $\frac{3}{10}$ mm. in diameter, delivering a drop equal to about 0.0075 c.c.

(67 drops per ½ c.c.). The pipette for the 1 per cent acetic acid solution is a 0.2 c.c. pipette graduated in 0.001 c.c.

Slide Holders (for three 3 × 2 inch slides).—The slide holder is a wooden lid of a slide box (3½ × 6¾ inches) containing an easily fitting thin wooden shelf having a small handle at each end. The holder for two slide holders

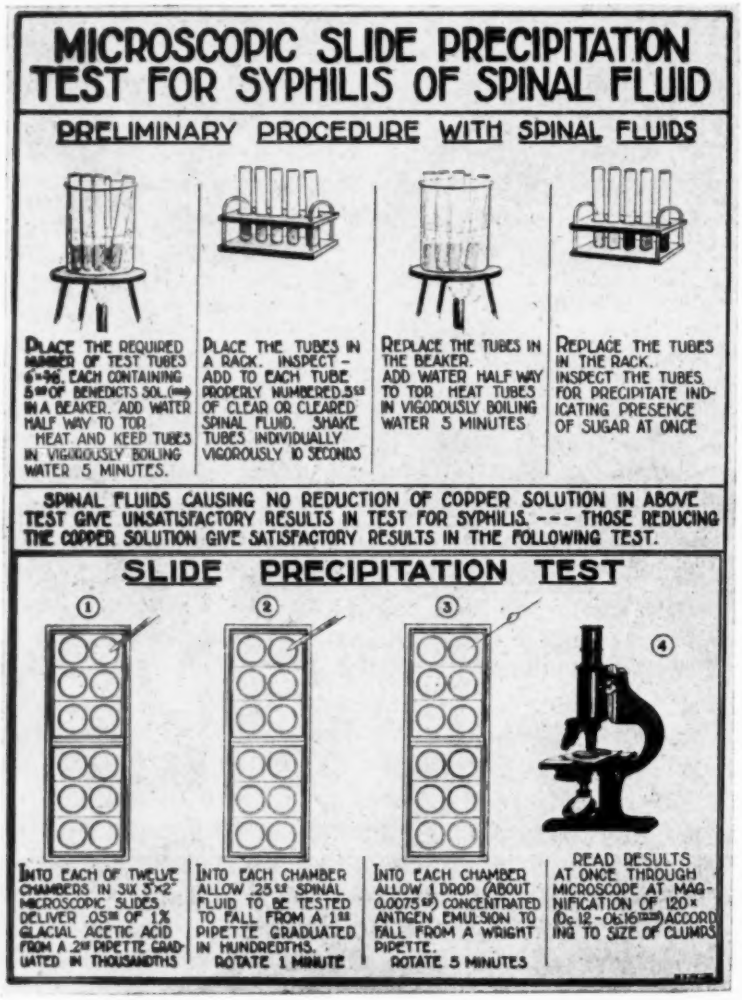
MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS OF SPINAL FLUID
PRELIMINARY PROCEDURE WITH SPINAL FLUIDS
PLACE THE REQUIRED NUMBER OF TEST TUBES 6×36, EACH CONTAINING 5cc OF BENEDICTS SOL. (cmp) IN A BEAKER. ADD WATER HALF WAY TO TOP. HEAT AND KEEP TUBES IN VIGOROUSLY BOILING WATER 5 MINUTES.
PLACE THE TUBES IN A RACK. INSPECT - ADD TO EACH TUBE PROPERLY NUMBERED .5cc OF CLEAR OR CLEARED SPINAL FLUID. SHAKE TUBES INDIVIDUALLY VIGOROUSLY 10 SECONDS
REPLACE THE TUBES IN THE BEAKER. ADD WATER HALF WAY TO TOP. HEAT TUBES IN VIGOROUSLY BOILING WATER 5 MINUTES
REPLACE THE TUBES IN THE RACK. INSPECT THE TUBES FOR PRECIPITATE INDICATING PRESENCE OF SUGAR AT ONCE
SPINAL FLUIDS CAUSING NO REDUCTION OF COPPER SOLUTION IN ABOVE TEST GIVE UNSATISFACTORY RESULTS IN TEST FOR SYPHILIS. --- THOSE REDUCING THE COPPER SOLUTION GIVE SATISFACTORY RESULTS IN THE FOLLOWING TEST.
SLIDE PRECIPITATION TEST
①
②
③
④
INTO EACH OF TWELVE CHAMBERS IN SIX 3"×2" MICROSCOPIC SLIDES DELIVER .05cc OF 1% GLACIAL ACETIC ACID FROM A 2cc PIPETTE GRADUATED IN THOUSANDTHS
INTO EACH CHAMBER ALLOW .25cc SPINAL FLUID TO BE TESTED TO FALL FROM A 1cc PIPETTE GRADUATED IN HUNDREDTHS. ROTATE 1 MINUTE
INTO EACH CHAMBER ALLOW 1 DROP (ABOUT 0.0075cc) CONCENTRATED ANTIGEN EMULSION TO FALL FROM A WRIGHT PIPETTE. ROTATE 5 MINUTES
READ RESULTS AT ONCE THROUGH MICROSCOPE AT MAGNIFICATION OF 120× (Oc. 12 - Ob. 16mm) ACCORDING TO SIZE OF CLUMPS

Fig. 1.

consists of a wooden lid (13⅝ × 3½ × ½ inches inside, 14½ × 4½ × 1 inches outside measurements) makes it possible to use six slides at one time. (See Fig. 1.)

One Per Cent Acetic Acid.—This solution is made just before use from glacial acetic acid. It is advisable to use no less than 1 c.c. of acid (delivered from a 1 or 2 c.c. pipette) and accordingly 99 c.c. of distilled water.

Antigen.—The antigen is a lipid obtained by precipitation in acetone at

50° C. to 37° C. of concentrated absolute alcohol extract of beef heart muscle powder (Difco). The details of its preparation are given in a previous report.[3]

Antigen Emulsion.—The antigen emulsion has the following formula:

0.85 c.c. Distilled water (P_H about 6.0) giving a lilac color with chlorphenol red indicator (LaMotte).

1.25 c.c. of 1 per cent cholesterin (Pfanstiehl c.p.) in absolute ethyl alcohol (99+ per cent). (The cholesterin solution is prepared in about

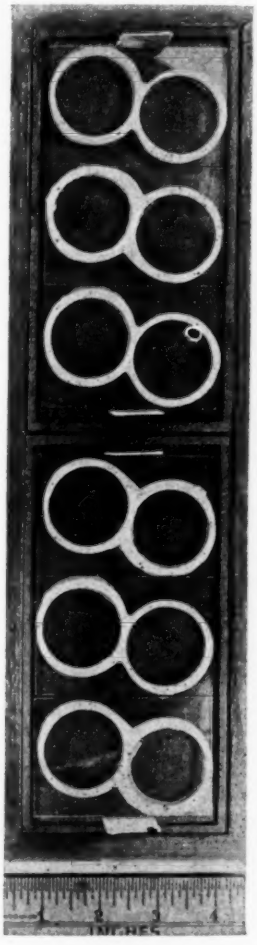

Fig. 2.

forty-five minutes by placing in an oven at 50° to 56° C. and shaking gently a few minutes at fifteen-minute intervals. Kept at 37° C. this solution is satisfactory for use for a few months).

0.1 c.c. Antigen.

2.2 c.c. of 0.85 per cent sodium chloride (c.p. or Reagent, Merck) solution (made with distilled water P_H about 6.0).

The emulsion is prepared as follows:

Into a one-ounce bottle 0.85 c.c. of distilled water is pipetted. The bottle

is held at an angle and the 1 per cent cholesterin in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds.

The bottle is held at an angle again, and 0.1 c.c. of antigen is pipetted against the side of the neck from a 0.2 c.c. pipette (graduated in thousandths).

The bottle is promptly stoppered with a cork and shaken vigorously (the fluid thrown from bottom to cork and back) for one minute.

The 0.85 per cent sodium chloride solution is then allowed to run in quite rapidly, the bottle is stoppered again and shaken fairly vigorously for one minute.

Allow the emulsion to stand one-half hour to one hour,* then centrifuge 2 c.c. at moderate speed for fifteen minutes, decant the supernatant fluid and with the tube inverted, dry the inner surface with a cloth almost to the level of the sediment. To the sediment add 0.5 c.c. of 0.85 per cent sodium chloride solution.

The emulsion when examined through the microscope, at a magnification of about 120 times, shows numerous very fine particles but no clumps whatever. Kept at room temperature, the emulsion is satisfactory for use for twenty-four hours.

The microscopic slide precipitation test for syphilis with spinal fluid described above represents the best method developed in a study of over 2800 spinal fluid specimens obtained through the courtesy of Dr. L. J. Karnosh, Dr. J. A. Toomey, Dr. I. R. Cohn, Dr. D. P. Seecof, and Dr. S. H. Rosin of City Hospital, Dr. A. R. Moritz of Lakeside Hospital, Mr. A. Reich of the Cleveland Clinic, Dr. H. J. Knapp and Mr. E. R. Miller of the Public Health Department, and Miss B. A. Burhans of Babies and Children's Hospital of Cleveland. We also wish to acknowledge here the assistance of Dr. O. Rimson with some of the preliminary experiments of this study.

Table I shows a clinical evaluation of the microscopic slide precipitation test and Wassermann tests with spinal fluids.

Table I shows the excellent agreement of the various tests with the clinical condition of the patients and furthermore shows the precipitation test to agree more closely with the clinical findings than the Wassermann test with the same antigen.

Table II shows a comparison of the microscopic slide precipitation test and Wassermann tests of spinal fluid with each other.

COMMENT

The microscopic slide precipitation test for syphilis with spinal fluid is based upon the principles of the test with serum.[4]

Since syphilitic spinal fluids ordinarily contain less reacting substance than syphilitic bloods, a larger quantity of spinal fluid than serum is tested. Furthermore, a quantity of acetic acid is added sufficient to assist in the pre-

*It has been found preferable since to use double the quantities in the formula above and to place 4 c.c. in each of two narrow test tubes (12 mm. inside diameter) placing one in a water bath at 35° C. for 15 minutes and the other in a water bath at 45° C. for 15 minutes after which they are treated as outlined above. One c.c. of 0.85 per cent sodium chloride solution is added to the sediment of each tube. The emulsion heated at 35° C. is used for a diagnostic test and the emulsion heated to 45° C. is used for an exclusion test.

TABLE I

CLINICAL EVALUATION OF THE MICROSCOPIC SLIDE PRECIPITATION TEST AND WASSERMANN TESTS OF SPINAL FLUID

	AGREEMENT									DISAGREEMENT									TOTAL TESTS
		SYPHILIS ± TO ++++ REACTIONS									SYPHILIS FALSE NEGATIVES							FALSE POSITIVES	
	NEGATIVE REACTIONS	PARESIS	TABOPARESIS	TABES	MENINGO-ENCEPHALITIS	C. N. S. UNCLASSIFIED	TOTAL C. N. S.	SYPHILIS OTHER THAN C. N. S.	TOTAL TESTS AGREEMENT	TOTAL TESTS DISAGREEMENT	SYPHILIS OTHER THAN C. N. S.	TOTAL C. N. S.	PARESIS	TABOPARESIS	TABES	MENINGO-ENCEPHALITIS	C. N. S. UNCLASSIFIED		
Microscopic slide precipitation test	768 76.3%	123	9	13	7	48	200 19.9%	11 1.1%	979 97.3%	27 2.7%	20 2.0%	6 0.6%		1	1		4	1 0.1%	Tuberculous Meningitis	1006 211 Syphilitic ± to ++++ = 21.0%
Mt. Sinai Wassermann test with the same antigen	456 74.6%	71	6	12	4	26	119 19.5%	10 1.6%	585 95.7%	26 4.3%	12 2.0%	13 2.1%	1	1	1		10	1 0.2%	Convalescent epidemic meningitis	611 129 Syphilitic ± to ++++ = 21.1%
Outside Wassermann test	596 76.0%	91	6	12	4	37	150 19.2%	19 2.4%	765 97.6%	19 2.4%	5 0.6%	12 1.5%	1	2	1		8	2 0.3%	1. Cerebral thrombosis 1. Chronic alcoholism, fracture (?) skull	784 169 Syphilitic ± to ++++ = 21.6%

21 histories unobtainable or cases doubtful.

± to ++++ any test in syphilitic serum = agreement.

Negative test in syphilitic serum, other test ± to ++++ = false negative.

++ to ++++ any test in non-syphilitic serum = false positive.

TABLE II

SEROLOGIC COMPARISON OF MICROSCOPIC SLIDE PRECIPITATION TEST AND WASSERMANN TESTS OF SPINAL FLUID

MICROSCOPIC SLIDE PRECIPITATION TEST AND MOUNT SINAI WASSERMANN TEST (SAME ANTIGEN)			MICROSCOPIC SLIDE PRECIPITATION TEST AND OUTSIDE WASSERMANN TEST		
AGREEMENT	RELATIVE AGREEMENT	DISAGREEMENT[1]	AGREEMENT	RELATIVE AGREEMENT	DISAGREEMENT[2]
566 92.3%	34 5.6%		733 93.1%	25 3.2%	
600 97.9%		13 2.1%	758 96.3%		29 3.7%

Evaluation of results:—
Positive Reaction = ++++, +++, and ++.
Doubtful Reaction = + and ±.
Agreement = positive, negative or doubtful by both methods.
Relative agreement = positive or negative by one method and doubtful by the other.
Disagreement = positive by one method and negative by the other, and vice versa.

[1]Disagreements:
8 positive Slide tests, negative Wassermann tests.
3 negative Slide tests, positive Wassermann tests.
1 false positive Slide test.
1 false positive Wassermann test.

[2]Disagreements:
11 positive Slide tests, negative Wassermann tests.
16 negative Slide tests, positive Wassermann tests.
2 false positive Wassermann tests.

cipitation reaction, but insufficient in itself to cause any precipitation of the antigen. Lastly, open slide chambers of optimal proportions are employed since results of reactions in them of syphilitic fluids are much more positive than when comparatively narrow test tubes are used.

CONCLUSION

The microscopic slide precipitation test for syphilis with spinal fluid described above is somewhat more sensitive than the Wassermann test with the same antigen and much simpler in detail, requiring no titrated hemolytic system of cells, amboceptor and complement. Furthermore, it requires no concentration of the globulins of the spinal fluid, it is performed with antigen emulsions that are satisfactory for use for twenty-four hours and the results of the reaction magnified about 120 times by the microscope are read accurately with ease.

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A SLIDE METHOD OF TITRATING BLOOD-GROUPING SERA*

By Arthur F. Coca, M.D., New York City, N. Y.

IT IS obviously of importance in emergencies in which a transfusion of blood is demanded within the shortest possible time that the test sera with which the groupings are done shall be of the highest agglutinating power obtainable in order that the results of a grouping test may be known within a few minutes.

For a number of years I have followed the practice of titrating the sera of the group A and group B students in my classes and have obtained the sera showing the highest titer for grouping purposes in the New York Hospital. Through this experience has been developed the convenient method of titration about to be described.

Two dilutions, 1-2 and 1-4, are made of each serum. A single cell-suspension of the corresponding cells is made by mixing one part of the whole defibrinated blood with an equal part of physiologic saline solution or by adding three parts of physiologic saline to one part of corpuscular sediment obtained by centrifugation.

Capillary pipettes are drawn out of Jena or Pyrex glass tubing (6 to 7 mm. outside diameter) the tips being of such a caliber that one hundred drops of saline (pipette in vertical position) will measure about 2.5 c.c. With such pipettes one drop of the 1-2 serum dilutions is mixed separately on an ordinary glass slide with one drop of the 1-4 blood cell suspension. Only two such tests should be made at one time on the same slide. An assistant is needed to note the time interval required for the first appearance of macroscopic clumping measured from the moment when the drop of serum and drop of cell suspension have been thoroughly mixed (with a knife blade) and spread over a circular area about 1.5 cm. in diameter. The clumping must be observed by looking down through the well-illuminated slide which should be held over a white surface. The mixtures are kept in motion by rocking the slide to and fro; cover glasses are not desirable.

If clumping occurs with the 1-2 serum dilution within one minute this result indicates that the respective serum is satisfactory for use as a grouping test serum. If under these conditions, clumping does not take place within one minute, the serum is discarded.

The method just described has been used in the Blood Donor Bureau of the Blood Transfusion Betterment Association, in New York City.

Among 251 Group A (anti-B) sera, 95 have been found to possess an agglutinating power for AB cells so high as to make the individuals from whom the sera are obtained dangerous as donors for Group AB recipients.

*From the Blood Transfusion Betterment Association of New York City and Cornell University Medical College.

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This relation has been revealed in the routine test of "quantitative compatibility" of Group A donors for Group AB recipients. The same test is made of the quantitative compatibility of Group O individuals that are offered as "Universal Donors" to patients of the other groups. A report upon the use of such donors will be made by Ella F. Grove.

Out of the 95 "dangerous" A individuals only 18 were further examined for high titered sera and among these, 4 were found whose serum in a dilution of 1-10 caused clumping of an equal volume of B cells diluted 1-4 on the slide in less than one minute.

Serum titrations with the slide method have been carried out on only 12 of our professional Group B donors in the Bureau. The serum of two of these individuals diluted 1-10, clumps an equal volume of the 25 per cent suspension of A cells on the slide in less than one minute.

I have extended the foregoing method somewhat in applying it to ten anti-A sera received from another source. The results of these tests are shown in Table I.

TABLE I

SLIDE TITRATION OF ANTI-A (GROUP III) SERA

	SERUM DILUTION	A CELLS DILUTION	TIME TO FIRST MACROSCOPIC CLUMPING
Grade I.			
Serum from Donor Bureau	1-4	1-4	11 sec.
Grade II.			
231)	1-2	1-4	14 sec.
230)	1-2	1-4	17 sec.
232)	1-2	1-4	25 sec.
235)	1-2	1-4	40 sec.
Grade III.			
237)	1-2	1-8	13 sec.
234)	1-2	1-8	15 sec.
233)	1-2	1-8	55 sec.
228)	1-2	1-8	59 sec.
Grade IV.			
236)	1-2	1-16	1 min. 27 sec.
229)	1-2	1-16	3 min. Still negative
231)	1-4	1-4	1 min. 20 sec.
230)	1-4	1-4	2 min. 46 sec.
232)	1-4	1-4	1 min. 20 sec.
235)	1-4	1-4	1 min. 57 sec.
237)	1-2	1-4	1 min. 40 sec.
234)	1-2	1-4	1 min. 42 sec.

Grade I and Grade II sera may be considered suitable for use as test sera. In this connection it may be pointed out that some Group AB corpuscles are distinctly less rapidly clumped by one or the other test sera than are the A cells or the B cells respectively. For this reason the Grade III and Grade IV sera should not be used.

In our experience the isoagglutinating sera keep best in a sterile condition *without preservative*. The two sera that are being used in the donor

bureau of this association have been under observation too short a time to allow judgment as to the rate of deterioration under the conditions mentioned.

In the fresh state both sera were in Grade I, clumping in about eleven seconds. Two months later the anti-B serum clumped in thirty-five seconds but the anti-A serum still clumped in about eleven seconds.

The grouping sera offered by three commercial biologic houses have been obtained out of the open market and have been tested. The results of these tests are shown in Table II.

TABLE II

SLIDE TITRATION OF COMMERCIAL GROUPING SERA

	SERUM DILUTION	CELL DILUTION	AGGLUTINATION
1	Anti-A 1-2	1-16	Negative in 3 minutes; a few clumps seen with microscope
	Anti-B 1-2	(1-16	20 seconds
		(1-8	60 seconds
2	Anti-A 1-2	1-32	Negative in 3 minutes; very few clumps seen with microscope
	Anti-B 1-2	(1-16	55 seconds
		(1-8	1 minute 25 seconds
3	Anti-A 1-2	1-32	Negative in 3 minutes; a few clumps seen with microscope
	Anti-B 1-2	1-16	Negative 4 minutes by microscopic examination

It is seen that the anti-B serum of Company 1 is barely classed as Grade III; the anti-B serum of Company 2 is classed as Grade IV; all the other sera are in Grade IV and quite unsuitable for grouping purposes.

In view of the unusually large material from which the professional staff of the Blood Transfusion Betterment Association is able to select the highest titered grouping sera the association is inclined to offer these sera to the profession. Inquiry regarding this may be addressed to the Executive Office of the Association in care of the New York Academy of Medicine, 2 East 103rd Street, New York City.

SUMMARY

1. A convenient method of titration of isoagglutinating sera is described with which the highest titered sera may be selected as test sera for grouping purpose.

2. A standard of agglutinating power is laid down for such selection.

3. The grouping sera offered to the profession by three reputable commercial biologic houses have been examined and all of them were found to fall below the standard, most of them being quite unsuitable for grouping purposes.

A MECHANICAL STAINING DEVICE*

By G. L. Rohdenburg, M.D., New York City, N. Y.

A CONTINUING increase in the volume of histologic examinations necessitates either the employment of additional help, or the development of machines to do routine work. Being confronted by this condition several years ago, we discussed the possibilities with a manufacturer of laboratory apparatus. These discussions coupled with investigations made by the manufacturer in other circles, led to the development of an apparatus which automatically performs the routine of dehydration and embedding of tissues in paraffine.[1] We have now adapted this apparatus for the routine processes of staining.

The machine (Fig. 1) consists of a cabinet (*a*) containing the mechanism of the apparatus (a motor, an eight-day clock and a Geneva drive). In the front of the cabinet is a fiber disk (Fig. 2), this disk will be described later. On top of the cabinet is a carrier frame (*b*) from which hang covers for 12 1,000 c.c. Pyrex jars arranged in circular fashion. From one arm of this carrier frame (*c*) hangs a slide carrier with a capacity of 30 slides. At predetermined time intervals this slide carrier is automatically lifted from one jar and placed in the next one.

The fiber disk (Fig. 2) has on it a number of lines, each one of which represents a division of time. The finest lines indicate five minutes, those of medium thickness fifteen minutes, and the heavy lines one hour. With a suitable punch, notches are made at the edge of the disk for the time periods at which it is desired to have the mechanism work. The disk is fixed in position, the electric current turned on, the starting mechanism switched in, and the clock having been wound the mechanism commences to work. Let it be supposed that the slides are to stay in the first jar ten minutes. As the disk revolves to the designated time interval an activating cam claw falls into the notch at the edge of the disk. The motor starts immediately, lifts the carrier arm to the required height, turns it in an arc which is one-twelfth of the circle and lowers the carrier into jar No. 2. The movement is clockwise. As soon as the carrier has come to rest in jar two, the motor is automatically shut off. This performance is repeated until all of the manipulations have been carried out. When the last movement has been made the claw drops into a notch which is intentionally deeper than the other notches and the entire electric mechanism is automatically switched off.

Once the slide carrier has been put in place and the apparatus started, no further attention need be paid to the machine. For example, slides can

*From the Achelis Laboratory of the Lenox Hill Hospital.
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[1]Auto-Technicon. Empire Laboratory Supply Co. The credit for the development of this apparatus should be given to Drs. Gross and Goldblatt.

be put in just before leaving the laboratory at night and the following morning they are ready for mounting. Starting at 4:00 P.M., it is now possible to dehydrate tissue blocks so that they are ready for embedding at 10:00 A.M.; to cut the sections, stain them in the machine and at 4:00 P.M., start the next lot dehydrating. The apparatus needs no attention other than that given any piece of machinery, e.g., oiling. Evaporation is negligible.

The details of operating technic vary according to what is to be accomplished.

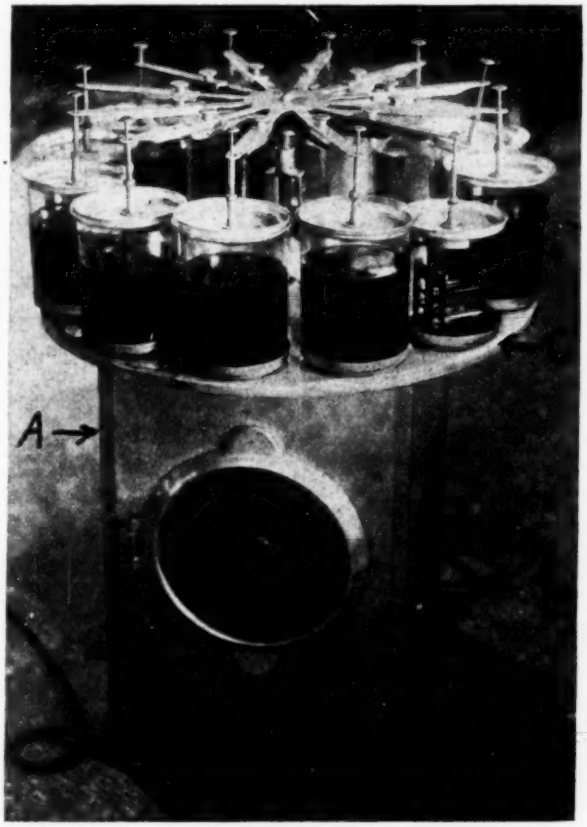

Fig. 1.

Method 1: The tissue is fixed in formalin for a period of twelve hours or longer, and cut on the freezing microtome. The sections are fished up on a glass slide previously coated with egg albumen and permitted to dry slightly, flushed with methyl alcohol and blotted with lintless blotting paper. The slide is then dipped for five seconds in celloidin (reduced to the viscosity of water) and air dried long enough for the celloidin to set. It is then placed in 80 per cent alcohol for a few moments and finally put in water. The slides are allowed to accumulate in the water until all the slides for the day have been prepared. Up to this point all slides have been handled individually. It will be recognized that this is the frozen section technic on fixed tissues. Inci-

dentally this method has the cheapest cost per block. From this point the method varies according to the number of slides.

From 1 to 30 slides. Select any jar as the starting point. In successive jars put 750 c.c. of the following: 1, Delafield's Hematoxylin diluted 1 part stain to 5 parts distilled water (other hematoxylin stains may be used, the degree of dilution being a matter of experiment); 2, tap water; 3, tap water; 4, tap water; 5, saturated alcoholic soluble eosin diluted 1 part stain to 7 parts 95 per cent alcohol; 6, 95 per cent alcohol; 7, 95 per cent alcohol; 8, synthetic methanol; 9, oil of origanum cretic; 10, oil of origanum cretic; 11, oil of origanum cretic; jar 12 remains empty.

Take the fiber disk and with the special punch make shallow notches at the following time intervals: 5 minutes; 20 minutes; 35 minutes; 45 minutes;

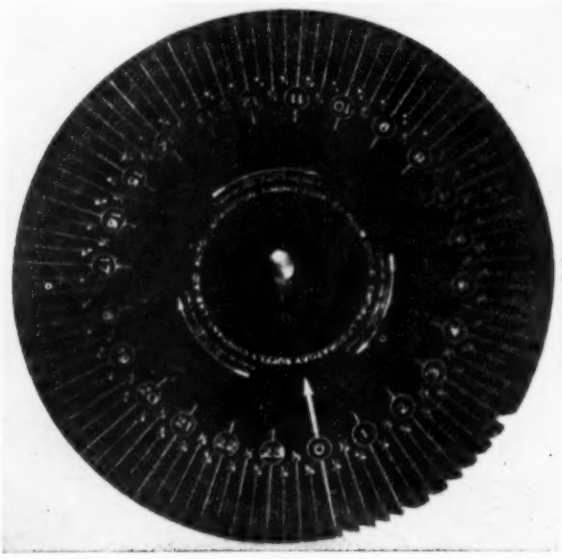

Fig. 2.

1 hour; 1 hour and 15 minutes; 1 hour and 45 minutes; 2 hours; 2 hours and 15 minutes; 2 hours and 35 minutes; a deep notch is made at 2 hours and 55 minutes.

From 30 to 360 or more slides. The only changes that need be made are (a) provide 12 slide carriers and 12 carrier arms, this will take care of 360 slides at a time. If the number is less reduce the number of carriers accordingly. (b) Arrange the punches on the fiber disk so that all the time intervals are equal (say fifteen minutes). Dilute the stains so that they stain properly in the time selected. This is a matter of experiment. Place the first carrier in the hematoxylin and when it moves to the first water, insert the second carrier into the hematoxylin. With each movement insert another carrier into the circuit. Removal of the carriers takes place in similar fashion. With such numbers the machine cannot be left unattended but it is possible

to take care of 660 slides in eight hours. This number can be tripled by increasing the size of the Pyrex jars and slide carrier.

Method 2: Paraffine sections are prepared. In rotation the jars are filled with the following solutions in the amount previously specified. Xylol; absolute alcohol; absolute alcohol; tap water; hematoxylin (diluted as per previous description); tap water; tap water; eosin (prepared as previously described); 95 per cent alcohol; 95 per cent alcohol; oil of origanum cretic; oil of origanum cretic. The time intervals for the disk are as follows: 10 minutes; 25 minutes; 40 minutes; 50 minutes; 1 hour; 1 hour and 15 minutes; 1 hour and 30 minutes; 1 hour and 40 minutes; 1 hour and 55 minutes; 2 hours and 10 minutes; 2 hours and 25 minutes; deep notch at 2 hours and 40 minutes.

Where special stains are done in large numbers, schedules can easily be devised to cover the specific instances; disks punched in accordance and filed away for use when necessary. Success is dependent upon proper dilution of the dye and this is solely a matter of experiment. Staining by this method, once the proper dilutions of the dye has been determined, is better and more uniform than that obtained with hand manipulation. The machine does not forget and it is never in a hurry.

THE DETERMINATION OF THE P_H OF PHOSPHATE BUFFER SOLUTIONS BY MEANS OF THE ANTIMONY-ANTIMONY OXIDE ELECTRODE*

By Russel J. Fosbinder, Philadelphia, Pa.

With increasing knowledge of the multiplicity of important rôles played by hydrions in biology, the biochemist is constantly searching for new methods which may facilitate the accurate determination of hydrogen ion activities. Any electrode, to be useful to the biochemist, should fulfill certain conditions, namely: (1) the absence of a gas phase is preferable, (2) it must not be easily poisoned or tend to catalyze certain reactions, (3) it must possess a reasonable degree of stability, and (4) the electrode should be as simple as possible.

A system, quite similar to the hydrogen electrode, is the metal-metal oxide electrode which has the advantage of not necessitating a gas phase. For this type a metal must be selected that does not replace hydrogen, whose salts are readily hydrolyzed, and whose oxide possesses stability and low solubility in both acid and basic solutions.

Uhl and Kestranek[1] first suggested the use of the antimony-antimony oxide electrode and employed it as an end-point indicator in titrimetric reactions. Subsequent investigators[2] have used the electrode with varying success. Roberts and Fenwick[6] in a very careful study have shown that the antimony electrode is completely reversible to hydrogen ions over a considerable P_H range.

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The purpose of the investigation presented in this paper was to devise a simple electrode system possessing a linear relation between the observed E.M.F. and P_H.

THE ANTIMONY-ANTIMONY OXIDE ELECTRODE

The cell reaction and potential respectively, corresponding to this electrode are

$$1/3\ Sb_{(s)} \rightleftarrows 1/3\ Sb^{+++} + (-)$$

$$E = E_o - \frac{RT}{nF} \ln a_{Sb^{+++}}$$

Considering the activity of the oxide and the activity of the water as constant and substituting the value of $a_{Sb^{+++}}$ in the former equation we have

$$E = E_0 + \frac{RT}{F} P_H \quad (P_H = p \propto H)$$

Experiments conducted in this laboratory using pure stick antimony in determining the P_H of phosphate buffer solutions yielded very unsatisfactory results. Electrodes prepared by a method similar to that employed by Brinkman and Buytendyk[3] gave results which were reproducible and constant as shown in Tables I and II. The electrode plating solution was prepared by dissolving pure antimony trichloride in Du Pont's glue, the glue being previously diluted with alcohol. After plating, the electrodes were treated with hot phosphate solution to remove excess chloride. It was also found advisable to keep the electrodes in a buffer solution until ready for use as the wet electrodes came to equilibrium in one minute, whereas six to eight minutes were required for the dry ones to reach a steady potential.

The phosphate buffers were prepared according to Sorensen from M/15 $Na_2HPO_4.2H_2O$ and M/15 KH_2PO_4 solutions. A temperature correction was applied where the solutions were used at 38° C.

TABLE I

$t = 24°$

ELECTRODE NUMBER	$P \propto H$ (SOLUTION)	$P \propto H$ (OBSERVED)	E (OBS.) VOLTS	E_{Sb} (E − 0.285) VOLTS	E′ (0.056 $P \propto H$)	E_0 (E_{Sb} − E′)	DEVIATION FROM MEAN MV
1	7.17	7.18	0.432	0.147	0.402	−.255	0.0
2	7.17	7.18	0.432	0.147	0.402	−.255	0.0
1	*7.38	7.38	0.443	0.158	0.413	−.255	0.0
2	*7.38	7.38	0.443	0.158	0.413	−.255	0.0
1	7.73	7.72	0.462	0.177	0.433	−.256	+1.0
2	7.73	7.70	0.461	0.176	0.433	−.257	+2.0
1	8.04	8.04	0.480	0.195	0.450	−.255	0.0
2	8.04	8.04	0.480	0.195	0.450	−.255	0.0

Mean = −.255
Average deviation = ±0.4

*Reference value.

The results contained in the tables were obtained from a study of the cell

$$Sb \mid Sb(OH)_3, (Buffer) \parallel N\ KCl, Hg_2Cl_2 \mid Hg$$

The data for Tables I and II were obtained at intervals of three to four days, freshly prepared buffer solutions being used for each set of determinations. In all, the electrodes were used over a period of more than two weeks. All electrodes prepared by the method used in this investigation gave E.M.F. values which agreed within one mv, provided they were allowed to stand in buffer solution for one day after their preparation.

TABLE II

$t = 38°$

ELECTRODE NUMBER	$P\alpha_H$ (SOLUTION)	$P\alpha_H$ (OBSERVED)	E (OBS.) VOLTS	E_{Sb} (E − 0.277) VOLTS	E′ (0.058 $P\alpha_H$)	E_0 (E_{Sb} − E′)	DEVIATION FROM MEAN MV
3	*7.41	7.41	0.482	0.205	0.430	−.225	+1.0
10	*7.41	7.41	0.482	0.205	0.430	−.225	+1.0
4	*7.41	7.41	0.482	0.205	0.430	−.225	+1.0
5	*7.41	7.41	0.481	0.204	0.430	−.226	+2.0
6	*7.41	7.41	0.482	0.205	0.430	−.225	+1.0
4	5.32	5.34	0.362	0.085	0.309	−.224	0.0
10	5.32	5.34	0.362	0.085	0.309	−.224	0.0
4	5.62	5.63	0.379	0.102	0.326	−.224	0.0
10	5.62	5.62	0.378	0.101	0.326	−.225	+1.0
3	5.93	6.02	0.401	0.124	0.344	−.220	−4.0
3	6.50	6.53	0.431	0.154	0.377	−.223	−1.0
5	6.50	6.53	0.430	0.153	0.377	−.224	0.0
3	6.84	6.84	0.449	0.172	0.397	−.225	+1.0
6	6.84	6.87	0.451	0.174	0.397	−.223	−1.0
4	6.84	6.85	0.450	0.173	0.397	−.224	0.0
10	6.84	6.83	0.451	0.174	0.397	−.223	−1.0
3	7.20	7.21	0.471	0.194	0.418	−.224	0.0
6	7.20	7.20	0.470	0.193	0.418	−.225	+1.0
3	7.20	7.22	0.471	0.194	0.418	−.224	0.0
3	7.76	7.74	0.501	0.223	0.450	−.227	+3.0
6	7.76	7.72	0.500	0.222	0.450	−.228	+4.0
5	8.07	8.08	0.520	0.243	0.468	−.225	+1.0
3	8.07	8.08	0.521	0.244	0.468	−.224	0.0
6	8.07	8.07	0.520	0.243	0.468	−.225	+1.0
10	8.07	8.08	0.521	0.244	0.468	−.224	0.0

Mean = −.224
Average deviation = ±1.0

*Reference value.

The value of E_0 obtained at 24° C., namely, −0.255 volts, differs considerably from that obtained by Roberts and Fenwick,[6] indicating that the electrode equilibrium probably involves a number of antimony oxides.

Plotting $P\alpha_H$ values as ordinates against values of E as abscissae, one obtains curves which are straight lines, indicating a linear relationship between E and $P\alpha_H$ in the range of hydrogen ion activity studied.

The relation between E and P_H obtained from the curves may be expressed by the following equations

$$E_{24°} = 0.030 + 0.056\ P_H$$
$$E_{38°} = 0.053 + 0.058\ P_H$$

A comparison of the values of the coefficients obtained for the antimony electrode with those for the hydrogen electrode reveals the high reversibility of the electrodes prepared by the method previously described.

TABLE III

TEMPERATURE	K (HYDROGEN)	K (ANTIMONY)	PER CENT REVERSIBLE
24° C.	0.059	0.056	95
38° C.	0.0617	0.058	94

Attempts to determine the P_H of whole blood using the method suggested by Brinkman and Buytendyk[3] were unsuccessful as a gradual drift in potential was observed.* This was also the case using diluted or undiluted plasma.

SUMMARY

Antimony electrodes, prepared by a simple procedure, have been used to determine the P_H of phosphate buffers.

The high degree of reversibility and reproductibility permits the determination of P_H values with an accuracy of ± 0.01 P_H.

Apparently the antimony electrode is not applicable for the determination of the P_H of blood.

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*Some time after the completion of this work Westenbrink, Pieters, and Pieters: Nederland. Tydschr. Geneeskunde 73:1, 2937, 1929 reported unsatisfactory results with blood.

CHART FOR COMPUTATION OF DAILY BASAL METABOLISM AND PERCENTILE BASAL METABOLIC RATE FROM SPIROMETER DATA*

By Curtis Bruen, M.D., New York City

THE basal metabolism determined by the spirometer method[1, 2, 3, 4, 5, 6] may be computed from the equation

$$C = 1440\ v\ \frac{273}{273 + t}\ \frac{p - 0.80\ p_w}{760}\ \frac{4.774}{1000}$$

in which C = calories per day, v = cubic centimeters volume decrement per minute, t = temperature in degrees centigrade, p = millimeters of mercury atmospheric pressure, and p_w = millimeters of mercury saturated aqueous vapor tension at temperature t. The daily basal metabolism is computed because it is in itself a clinically significant quantity. The cubic centimeters minute volume decrement, the volumetric contracture of the spirometer bell during the determination divided by the number of minutes of its duration, is multiplied by 1440 to raise it to the daily volumetric change under the temperature, aqueous vapor tension, and atmospheric pressure of the determination. This volume is reduced to standard conditions, dry. The aqueous vapor is computed as 80 per cent saturated. Soda lime of maximum carbon dioxide absorption power contains 4.5 per cent alkali and 18 per cent water. With soda lime of this alkali content, the relative vapor pressure increases very slowly with temperature, and at 25° C. approaches 80 per cent with water content above 15 per cent.[7] Soda lime of 4.5 per cent alkali content and 16 to 18 per cent water content maintains this relative humidity in the spirometer substantially constant at all temperatures.[8, 6] The corrected volume is multiplied by the per liter calorific value of oxygen divided by 1000. In the fundamental series of determinations on normal men and women[9] the total respiratory quotient averaged 0.82.[10, 3] The normal basal protein metabolism is taken as 15 per cent of the total calories. At the given respiratory quotient oxygen produces 4.774 calories per liter** with this rate of protein metabolism. The basal metabolism de-

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**Calculated[11] from the factors

	Respiratory Quotient	Calories per Liter O_2
Protein	0.802	4.463
Fat	0.707	4.686
Starch	1.000	5.047

by means of the equations

$$0.82 = 0.15\ (0.802) + 0.85\ R.Q.\ non\text{-}P.$$

$$R.Q.\ non\text{-}P. = \frac{0.82 - 0.15\ (0.802)}{0.85}$$

$$cal./l.\ O_2 = 0.15\ (4.463) + 0.85\left(4.686 + (5.047 - 4.686)\ \frac{\frac{0.82 - 0.15\ (0.802)}{0.85} - 0.707}{1.000 - 0.707}\right)$$

termined is compared with a normal standard for the subject on the basis of plus or minus percentile difference.*

Computation schedules,[2, 4, 12, 13, 14, 15] tables,[13, 14, 16, 8, 17, 18, 6, 19] slide rules,[20, 21] and charts [22, 23] are available for making these calculations. The present method is distinctive in obtaining the daily basal metabolism directly from the spirometer data by locating two coordinates, sighting their intersection, and reading its value, and indicating the plus or minus percentile basal metabolic rate by measuring on scales the distance between the determined basal metabolism and the standard. The x and y axes are divided in logarithmic units. A scale of minute volume decrement‡ is plotted on the x axis from the equation

$$x = \log v.$$

On a series of equidistant isotherms parallel to the y axis scales of atmospheric pressure are plotted from the equation

$$y = \log 273 - \log (273 + t) + \log (p - 0.80\ p_w) - \log 760.$$

Isobars are drawn incorporating them in a grid. A series of graphs and a scale are plotted from the equation

$$\log C - \log 1440 - \log 0.004774 = x + y.$$

Parallel equidivisional scales of the respective proportional parts, the successive multiples of which approximate the logarithms of the successive plus and minus percentages over the maximum range, are plotted. Auxiliary scales of the exact logarithms of plus and minus percentages beyond these limits are plotted for correcting readings beyond the range of the equidivisional scales.

Chart I computes daily basal metabolism from minute volume decrement, average spirometer temperature, and atmospheric pressure, and measures this against a standard in terms of plus or minus percentile basal metabolic rate.

Spot the cubic centimeters minute volume decrement, carry a line vertically into the field of the chart, spot the average temperature in degrees centigrade, carry a line vertically into the network, spot the millimeters of mercury atmospheric pressure, carry a line into the network in the direction of the guide lines to its intersection with the vertical line previously traced, from this point carry a line horizontally into the field of the chart to its intersection with the vertical line previously traced, from this point carry a line in the direction of the graphs to the scale of calories, read the result, spot the normal basal metabolism for the subject computed from the companion charts

*By convention oxygen is assigned the calorific value it would have if the total respiratory quotient were a nonprotein respiratory quotient. Allowing for basal protein metabolism of 15 per cent total calories reduces the per liter calorific value of oxygen from 4.825 to 4.774 with the respiratory quotient of 0.82 current in this country. The computed basal metabolism is as a result diminished 1.06 per cent.

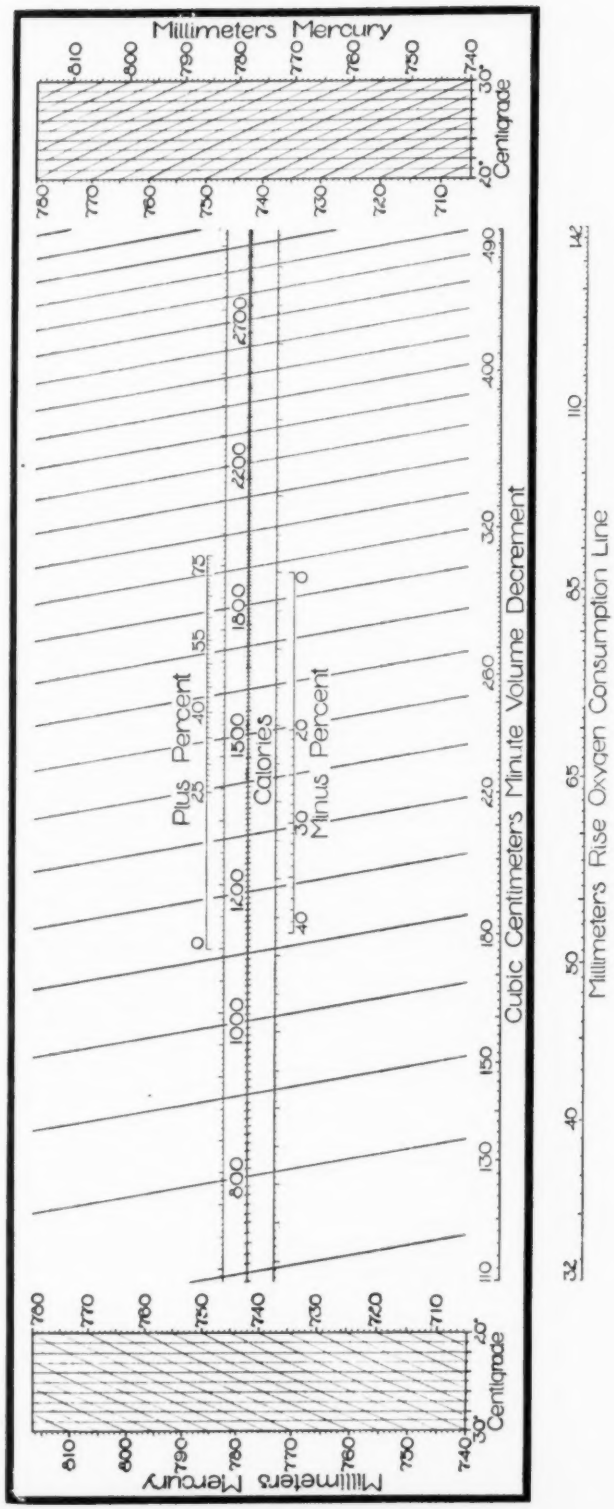
‡This scale may be plotted or regraduated for any standardized apparatus and technic. For a spirometer bell with a contracture or 20.73 c.c. per mm. and a six-minute test period[6] the scale equation is

$$x = \log \frac{20.73c}{6}$$

and the conversion equation would be $c = \frac{6\ v}{20.73}$

in which c = six-minute contracture in millimeters.

Such a substitutive scale of millimeters rise of the oxygen consumption line applicable to the graphic modification of this apparatus is plotted below the chart proper.


Millimeters Mercury
Centigrade
Plus Percent
Calories
Minus Percent
Cubic Centimeters Minute Volume Decrement
Millimeters Rise Oxygen Consumption Line

for the Du Bois[24] or Harris-Benedict standards,[25] measure the distance between determined and standard values to the nearest per cent plus or minus on the proper equidivisional scale, if this reading is greater than plus 25 or minus 20 correct it by measuring it against the adjoining exact scale.

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A FURTHER MODIFICATION OF THE VARNEY ANAEROBIC JAR*

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THE principle of the Varney jar is based on the removal of free oxygen by the oxidation of white phosphorus.

The original Varney jar[1] consisted of a standard museum jar. In the modified form described by Damon[2] a galvanized iron can was used in place of the glass jar, the chief disadvantage of which lay in the possibility of its breakage. No improvement over the original jar was offered in regard to its internal equipment.

The object of this paper is to emphasize the importance attached to the selection of a suitable dish in which the oxidation of the phosphorus takes place. In my hands the glazed porcelain dish recommended originally gave unsatisfactory results, due to the collection of water within. Phosphorus pentoxide formed during the combustion of phosphorus has a hygroscopic action which possibly may be accelerated under the influence of the glazed porcelain dish. However, regardless of any catalytic influence it may have, the glazed porcelain dish favors the accumulation of water which interferes with the phosphorus oxidation before the establishment of effectual anaerobiosis. Nothing of this occurrence is indicated in the references cited.

It is suggested that culture dishes of either red or white earthenware be employed to obviate the unfavorable conditions resulting from the use of glazed porcelain. The earthenware affords an efficient absorbing surface to remove any deleterious moisture that is being formed. Both glazed and unglazed earthenware dishes are available. The former are unglazed inside but glazed on the outside. This reduces the absorptive ability to some extent but they have been used with good results. The earthenware dish should always be perfectly dry at the time of employment.

For the convenience of the reader a brief description is given of the construction of the jar as used by the writer, and the manner in which it has been successfully operated.

Construction.—The museum jar or the galvanized iron can which has a snugly fitting lid is approximately 6½ by 12 inches in size. A cylindrical frame, 11 inches in height and of such diameter as to be freely movable in and out of the can, is constructed of heavy steel wire. About one inch from the bottom of the frame a galvanized wire gauze platform is soldered to its sides. A cylinder, 8 inches in height and closed at one end, fits closely within the wire frame in an inverted position so that the open end comes to rest on the wire gauze platform. The diameter at the summit of the frame is flexible so that it can be spread apart to insert the cylinder, then rebounds to grasp

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it firmly but not enough to inconvenience its removal or replacement. Two asbestos discs, one having the diameter of the cylinder, the other a diameter exceeding that of the wire frame, and the culture dish of either red or white earthenware in which the phosphorus is oxidized, complete the outfit.

Operation.—About one-half inch of water for the absorption of fumes is poured into the bottom of the can. The cultures either on Petri plates or in tubes are placed on the wire gauze platform of the frame. The cylinder is next inverted and pushed down to about halfway the height of the frame. A methylene blue indicator, a description of which will follow, is heated in a test tube over a Bunsen burner until colorless and immediately placed with the cultures beneath the cylinder which is then lowered to the wire base. The frame, containing the cultures and indicator under the inverted cylinder, is placed within the can; the small disc of asbestos is then placed on top of the cylinder and the dry earthenware culture dish on the asbestos disc. One-half to one inch of a large stick of commercial white phosphorus is taken from a container of water, quickly touched to blotting paper to remove excess moisture and placed in the earthenware culture plate. To insure a rapid anaerobiosis, the phosphorus is ignited by a match (best done in a hood connected with good suction), the remaining disc of asbestos is quickly placed on the top of the wire frame which projects an inch above the phosphorus dish and the lid closed *immediately* and sealed air-tight with plasticine around the periphery. It is convenient for quick sealing to have the plasticine in place as a roll around the edge of the lid before the procedure of closing the can is begun. It is important during continuous operation of the can that precautions be taken to exclude all moisture, resulting from the previous use, from the immediate vicinity of the phosphorus oxidation site, e.g., from the asbestos covering and phosphorus dish itself. Good anaerobiosis was always obtained by strictly observing these precautions.

Indicator.—In order to be assured of anaerobiosis an indicator described by McIntosh and Fildes[3] is regularly employed. On each occasion of closing the can equal volumes of the following three solutions

(1) 6 c.c. of N/10 NaOH per 100 c.c. of water
(2) 3 c.c. of 0.5 per cent aqueous methylene blue per 100 c.c. of water
(3) 6 gm. of glucose per 100 c.c. of water with a small crystal of thymol added

are mixed in a test tube, boiled until the methylene blue is thoroughly reduced to a colorless state and placed immediately in the can which is then rapidly sealed in the manner described above. If a good degree of anaerobiosis is obtained the indicator will remain colorless until the subsequent opening of the can; if otherwise, the blue color will return, its intensity depending upon the amount of free oxygen available.

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A NOTE ON THE MICROSCOPIC DIAGNOSIS OF ACID-FAST ORGANISMS*

By Ruby L. Barnhart, A.B., and Frederick Eberson, Ph.D., M.D.,
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RECENTLY, modifications of the Ziehl-Neelsen method of staining tubercle bacilli have been introduced with a view to improving microscopic diagnosis of acid-fast bacilli. The changes have been concerned chiefly with the process of decolorizing and counterstaining. It has been found, for example, by Gardner[1] and Shunker[2] that a yellowish counterstain brings the tubercle bacilli into bolder relief. The methylene blue counterstain has proved less effective in this respect, owing to its tendency to produce a purplish or black mixture. This objection, it appears, has been met by a new staining technic described by Ransom.[3]

From these observations it might be inferred that an essential requirement for sharp definition of tubercle bacilli is a contrasting yellowish stain. Routine experience with various types of illumination has convinced us that equally satisfactory results are obtainable by simply utilizing a yellowish light and dispensing with daylight or the so-called "daylight" electric bulbs. Ordinary 50 to 75 watt plain or frosted bulbs are suitable for the illumination of the microscopic field. The Ziehl-Neelsen technic for staining need not be modified in any particular.

Examinations were made of acid-fast organisms in sputa, stools, pus, and material from lymph nodes with unequivocal results. The bacilli appeared brilliant red, contrasting markedly with the background. Identification of tubercle bacilli was exceedingly simple and reliable provided the source of illumination had some degree of yellowish tint.

CONCLUSION

The use of a yellowish source of illumination for the microscopic examination of material containing tubercle bacilli or other acid-fast organisms is suggested. Yellowish light brings acid-fast organisms into bold relief as brilliant red bodies that can be seen readily. This form of illumination is superior to that furnished by "daylight" bulbs or natural light. The reliable Ziehl-Neelsen staining technic need not be modified or abandoned if the suggested simple procedure is followed.

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DUST-SHIELD ON STILL FOR INTRAVENOUS SOLUTIONS*

By Richard B. Stout, M.D., Madison, Wis.

DURING the course of an investigation into the cause of mild reactions following intravenous therapy, it was decided to double-distill all water for the purpose.

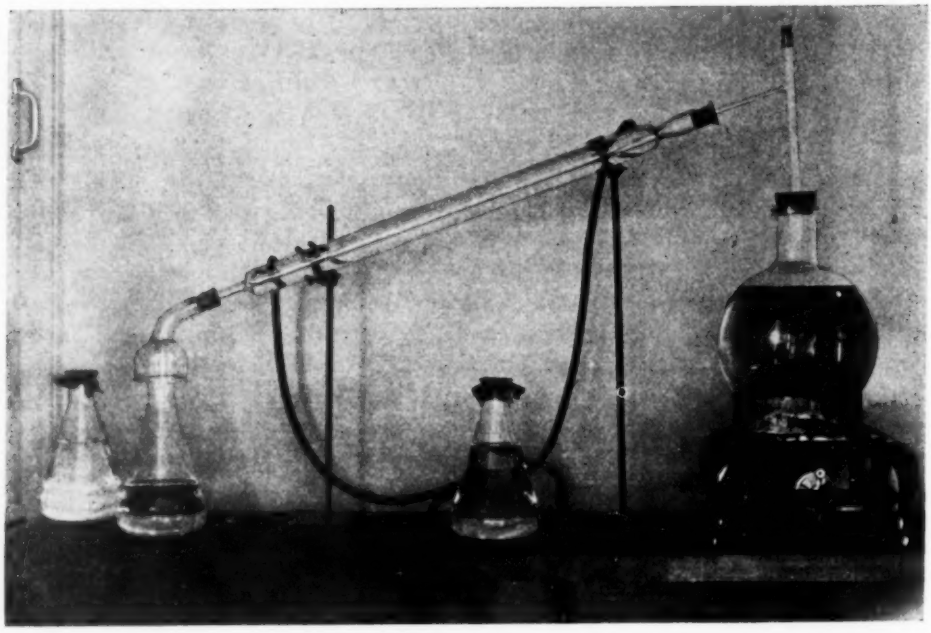

Fig. 1.—Still in operation.

A gas hot plate with a metal supporting ring, a five liter Pyrex balloon flask and a twenty-four inch Pyrex condenser were set up in a corner of the hospital laboratory.

To preclude the possibility of dust contamination of the distillate, a curved Pyrex delivery spout with dust-shield was made† similar in design to the tips used in bacteriology laboratories for filling sterile test tubes with sterile media.

Water is distilled into Pyrex flasks, saline or glucose added and the solution autoclaved before use.

*From the Surgical Section of the Jackson Clinic.
Received for publication, July 14, 1930.
†Made by the Milwaukee Glass Works, Milwaukee, Wisconsin.

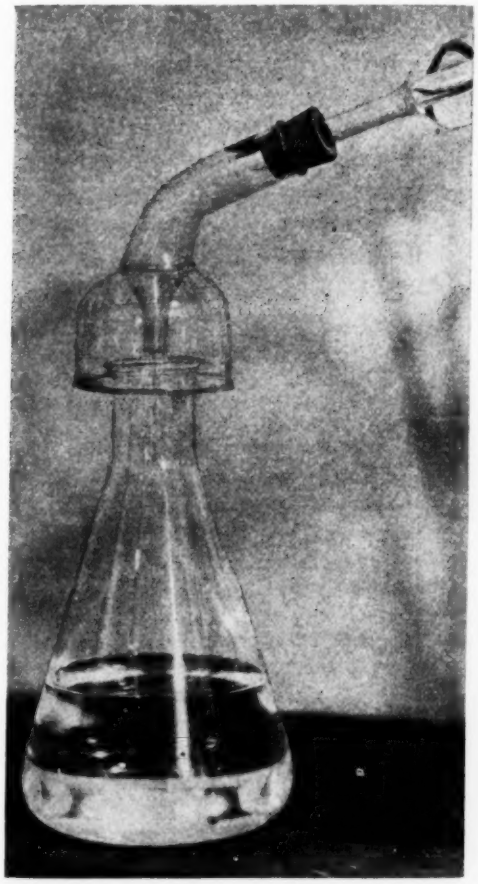

Fig. 2.—Dust-shield is attached to end of condenser by means of a rubber cork and conveys water to flask without possibility of dust contamination.

Solutions prepared in this manner have been free from foreign material and no reactions have followed their use.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUMOR: Diagnosis of Intracranial by Supra-Vital Technic, Eisenhardt, L., and Cushing, H. Am. J. Path. 6: 541, 1930.

The method is that described by Sabin (Sabin F. R. Studies of Living Human Blood Cells, Bull. Johns Hopkins Hosp. 34: 277, 1923).

Technic: The method is that described in detail by Sabin in her studies of living blood cells. Its application to the more solid tissue of intracranial tumors is quite simple.

As soon as the tumor is exposed at operation, a fragment of it is routinely submitted for immediate examination. A minute portion is separated with dissecting instruments and placed on a glass slide.

The slide may be previously prepared with an even film of neutral red dye, or a mixture of neutral red and Janus green, the exact directions for which are given by Sabin. Satisfactory results are obtained when a clean slide is used and a drop of aqueous solution of neutral red dye (1:10,000 or more) added directly to the tissue.

A cover glass is immediately placed over the tissue, which is carefully spread by gentle pressure on the cover glass, the rubber end of a pencil being convenient for the purpose. One learns by experience how much pressure to apply in making the smear. Some tumors are so soft that they spread almost like fluid, and anything more than the lightest touch damages the cells. Other tissues are very firm, fibrous or even gritty with calcium, requiring greater effort in flattening them out for a satisfactory film.

The preparation is sealed at once with a mixture of vaseline and paraffin around the edge of the cover glass, and is then examined microscopically in a warm box.

The actual preparation of the smear takes less than a minute.

There are one or two precautions which may be particularly commented upon at this point. If too strong a solution of the dye is used, the nuclei will be stained diffusely, a sign that the cells have been injured. The edges of the preparations are usually overstained in this manner. While overstaining does not necessarily so obscure a microscopic picture as to make a diagnosis uncertain, it must be avoided if one is interested in studying the cytological elements of these tumors.

Furthermore, the importance of making the preparations as soon as possible after the tissue has been removed should be emphasized whether or not an immediate microscopic report is imperative. A diagnosis may be made in most cases even though the smears are prepared an hour or several hours later, provided the tissue has not been allowed to dry. However, there is no question but that the cells undergo changes on long standing, especially if the tumor is of a soft consistency. They become swollen and vacuolated, the cytoplasm markedly granulated, and the nuclei show degenerative changes. When extreme vacuolization occurs one vacuole may fill the whole cell. In some areas cytoplasmic boundaries become unrecognizable, so that there is only disintegrated material to be seen.

In every case for comparison a portion of the same tissue is fixed to be subsequently sectioned and stained by the usual methods.

BLOOD PLATES: Indirect Method of Counting, Olef, I. Arch. Int. Med. 46: 585. 1930.

The following solution is required: Sodium metaphosphate (Merck) 2 gm., sodium chloride 0.9 gm., distilled water 100 c.c.

The method is as follows:

Two or three large drops of the sodium metaphosphate solution are placed in a small paraffin cup. The cup can be prepared by melting the center of a small cube of

paraffin, about 2 cm. on the side, with the heated end of a glass rod. A small drop of freshly drawn blood is allowed to fall into this solution, producing a dilution of approximately 1:10. This is gently stirred once or twice with an ordinary wooden applicator, the end of which is coated with paraffin. A few small drops or one large one of this mixture is transferred by means of the wooden applicator to a cleaned glass slide, covered with a cleaned cover slip and sealed with petrolatum. Two preparations should always be made and if the ratio of platelets to erythrocytes varies markedly in the two, new specimens should be prepared. An oil immersion lens should be used. A square diaphragm in the ocular facilitates counting; this can be made from thick paper or a card, and forms a simple substitute for Ehrlich's ocular. The platelets and erythrocytes are both counted in fields taken at random in different parts of the specimen, until at least from 250 to 500 red cells in each of the two preparations have been seen. A red cell count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined.

The results in normal individuals are higher than hitherto reported.

SEDIMENTATION TEST: In Tuberculosis, Banyai, A. L., and Anderson, S. V. Arch. Int. Med. **46**: 787, 1930.

The following modification of Westergren's method was employed:

Draw 3.8 per cent sterile sodium citrate solution to the 0.4 c.c. mark of a 2 c.c. syringe previously washed with the same solution, taking care to shake out all air bubbles. Draw blood either directly from the patient's vein or from a tube of fresh blood. Fill the syringe to 2 c.c., making the ratio of citrate to blood 1:5. Then allow a 0.1 c.c. bubble of air to enter, and invert the syringe several times permitting the bubble to travel back and forth, thus mixing the blood and citrate solution. The blood is now ready to be transferred into a sedimentation tube, which is a 1 c.c. clear glass container, open at both ends and graduated into 100 equal parts like the barrel of a Luer tuberculin fornier. Before the tube can be used it has to be sealed at the base with a mixture of equal parts of paraffin and petrolatum melted together. The wax is packed into the tube up to where the graduation begins. The tube is held upright in a large-sized rubber stopper. Let one or two drops of the blood mixture run first to the very bottom of the sedimentation tube, and the rest will follow easily without blocking the narrow opening. When the tube is full as nearly as possible to the 1 c.c. mark, record the exact time, and set the interval timer to ring in fifteen minutes, when the first reading is made. Other readings follow in half an hour (from the time the test was started), one, two and twenty-four hours.

The results are recorded on a graph on which the time is indicated along the horizontal line and the percentage reading of the red blood cell level along the vertical line. To make comparison simple, a minimal normal curve, based on the sedimentation rates of eighty healthy persons, is always shown on this graph. The readings of the normal curve are 97, 95, 90, 75, 40, at the time intervals above mentioned; readings above this series are, of course, normal too. For the sake of convenience and brevity, the whole series of readings may be stated as one figure which actually represents the percentage of a test as compared with the minimal normal curve. For example, add the figures 97, 95, 90, 75 and 40 given above and 3 (an arbitrary number) and divide the sum by 4; the result equals 100. Compare the normal with a test reading, 90, 75, 60, 50, 40 (add 90, 75, 60, 50, 40, 3 and divide by 4) the result is 79. In other words, this test is only 79 per cent of the normal.

From a study of 2000 cases the authors confirm the value of the test as an index of activity and suggest its application to determine working capacity before discharge.

TISSUE: A New Reticulum Stain, Pap, T. Centralbl. f. Allg. Path. u. Path. Anat. **47**: 116, 1929.

The method is used after formalin fixation.

1. Aqueous, 0.25 per cent potassium permanganate; for from five to eight minutes.
2. Rinse in distilled water for from fifteen to twenty seconds.
3. Oxalic acid, 5 per cent, for from eight to ten minutes.
4. Rinse in three changes of distilled water for one minute.

5. Aqueous, 2 per cent, silver nitrate for twenty-four hours.
6. Wash in two changes of distilled water.
7. Ammoniacal silver nitrate, 1 per cent, for one-half hour. (Note: 1 grain [0.065 gm.] of silver nitrate is dissolved in 10 c.c. of distilled water to which 11 drops of 40 per cent sodium hydroxide are added. The precipitate formed is then dissolved by the addition of sufficient 26 per cent ammonium hydroxide and the solution made up to 100 c.c. with distilled water.)
8. Wash in two changes of distilled water.
9. Immerse in 4 per cent neutral formaldehyde for from five to fifteen seconds.
10. Wash in tap water.
11. Gold chloride solution, 0.2 per cent, five minutes.
12. Wash in tap water.
13. Sodium thiosulphate, 5 per cent, for from five to ten minutes.
14. Wash in tap water for one minute.
15. Dehydrate in series of alcohols.
16. Chloroform.
17. Mount.

BOLTZ TEST: In Urinalysis. Brice, A. T. Arch. Int. Med. 46: 778, 1930.

The technic is as follows:

a. Place in a small glass test tube 1 c.c. of the filtered or centrifuged specimen to be examined.

b. Add 0.3 c.c. of acetic anhydride, preferably a brand high in aldehyde content, from a pipette, drop at a time, with shaking.

c. Add 0.8 c.c. of concentrated sulphuric acid, from a pipette, a drop at a time, with shaking.

d. Let the tube stand at room temperature under observation for five minutes. The positive reaction is indicated by the appearance of a light lilac to deep purple color. Occasionally, in weak positive reactions, the positive color may appear within the five minute period and subsequently be obliterated by the darker colors due to the reaction of other substances in the urine. Occasionally, in strongly positive reactions the positive color may not appear before the expiration of some minutes, owing to the darker colors produced immediately by the reaction of other substances in the urine. In strongly positive specimens the purple color may be so dark as to require dilution of the preparation with water in order to differentiate the purple from possible dark browns or blacks. The prescribed five minutes of the test is a valid test period, as positive specimens have been shown to hold the positive color for several hours. The test with urine is practically impossible to read by any of the artificial lights with which I have experimented up to the time of writing.

ENTAMOEBA HISTOLYTICA: Recent Improvement in the Cultivation of, Cleveland, L. R., and Collier, J. Am. J. Hyg. 12: 606, 1930.

The authors have recently improved cultural methods so that it is possible to start a culture from a single organism. The following medium is spoken of as "virtually specific" for E. histolytica.

It consists of liver infusion agar slants covered with serum-saline with the addition of a small amount of sterile rice flour. The method of preparation follows:

The liver infusion agar is prepared by the Digestive Ferments Company of Detroit, Michigan, and each pound contains an infusion from 500 gm. of beef liver, 10 gm. of peptone, 5 gm. of sodium chloride, and 20 gm. of agar. The best results have been obtained by using 30 gm. of this and 3 gm. of Na_2HPO_4 per liter of distilled water instead of 55 gm., the amount called for in the directions of the Digestive Ferments Company. This makes a softer slant and one not so rich. This medium can be prepared as easily as any agar slant medium, and no difficulty is met in autoclaving, as sometimes happens in the sterilization of egg or serum slants. After sterilization the slants are covered with fresh horse serum-

saline (0.8 per cent), (1-6) and a 5 mm. loop of sterile rice flour is added to each tube. The authors have found rice flour or powdered unpolished rice superior to pure rice starch and have used it in all media.

When a tube of this liver infusion serum-saline medium is inoculated with a 5 mm. loop of amoeba in blood and mucus from a fresh stool, the amoeba begin to grow very quickly, and within forty-eight to seventy-two hours become fairly numerous, a good number of cysts nearly always being formed by the end of seventy-two hours, and, in a few instances, as early as forty-eight hours. If a subculture is made after forty-eight hours or seventy-two hours, a very abundant growth is obtained within two to three days, the amoeba becoming practically as numerous in the rice flour and débris at the base of the slant as blood cells are in the blood stream. Very often from 10 to 50 per cent of the amoeba are encysted within two to three days after the first subculture. When subcultured, these cysts, if mature, excyst within four to six hours; and, if not mature, they continue development until mature and then excyst. In each subculture there are usually a few cysts present at the end of forty-eight hours, but not very many because in this medium excystation, metacystic development, and rapid multiplication of trophozoites occur simultaneously. Subcultures are made with a sterile wide mouth pipette made from glass tubing. The reason for the wide opening in the pipette is that it is necessary in making subcultures to pick up with the pipette 1 to 3 drops of the rice flour and débris at the bottom of the tube with little or no fluid. This is really the only precaution necessary to keep the amoeba growing abundantly for as long a period as one desires. If good cultures are wanted, the authors emphasize strongly the necessity of removing only the solid material from the bottom of the tube. Of course, the amoeba will grow if 4 or 5 drops of liquid are removed with the solid matter at the time of subculture, but the difference in growth and activity of the amoeba is certainly great enough to warrant the little extra care necessary to remove only the solid material. The best growth is obtained by subculturing the five or six drops of practically solid material from one tube to two or three tubes every forty-eight hours.

HEMOFLAGELLATES: Cultivation and Differentiation of, Cleveland, L. R., and Collier, J. Am. J. Hyg. **12**, 614, 1930.

Nineteen formulas are described as follows:

1. In preparing the Salle and Schmidt medium certain modifications are convenient, as follows:

"Difco" veal infusion	250 c.c.
Proteose peptone	10 gm.
Sodium chloride	5 gm.
Distilled water	550 c.c.

After autoclaving, add 20 c.c. of 50 per cent glucose sterilized by filtration and 60 c.c. horse cells laked with 2 parts of distilled water.

2. a. Dissolve proteose peptone, 1 per cent, sodium chloride 0.8 per cent, and glucose 1 per cent in distilled water and autoclave.

b. Lake horse cells in sterile distilled water, 2 parts of water to 1 of cells.

Mix 6 parts of a to 1 part of b.

3. Lake horse cells with distilled water (1-3), autoclave, filter through cotton, measure, and then add enough of each of the following to make the mixture 0.8 per cent sodium chloride, 1 per cent glucose, and 1 per cent proteose peptone. Tube and autoclave.

4. This is the same as medium 3 except that 1.5 per cent of peptonized milk is used instead of the peptone and glucose.

5. Mix equal parts of medium 3 and Difco liver infusion agar (removing agar before use) and add glucose to make 1 per cent. Tube and autoclave.

6. Dissolve 60 gm. of Difco whole blood in 1000 c.c. of distilled water, autoclave, filter through cotton, measure, and add enough of the following to make the mixture 0.8 per cent NaCl, 1 per cent proteose peptone, and 1 per cent glucose.

7. Dissolve 15 gm. of Difco special hemoglobin in 1000 c.c. of 0.8 per cent NaCl and add glucose and proteose peptone to make 1 per cent of each. Tube and autoclave.

8. Dissolve 60 gm. of Difco special hemoglobin in 1000 c.c. of 0.8 per cent NaCl, autoclave, filter through cotton, measure, and add glucose and proteose peptone to make 1 per cent of each. Tube and autoclave.

9. Make a paste of 25 gm. of Difco special hemoglobin in 250 c.c. of distilled water. Add 150 c.c. of N/1 NaOH to the paste, stir well, and incubate at 37° C. for forty-eight hours. Then adjust the hydrolyzed hemoglobin to P_H 7.6 or 7.8 with 5 per cent HCl (usually about 50 c.c.) and add distilled water to make the salt content approximately 0.8 per cent (usually about 456 c.c.). Now, add sufficient proteose peptone and glucose to make the mixture 1 per cent of each. Tube and autoclave. The only precaution necessary in preparing this medium is not to allow the P_H to drop to 7.0 or lower while the medium is being autoclaved, because, if this happens, precipitation will occur. The Digestive Ferments Company has prepared this medium in a dehydrated form and all that is necessary in using it is to dissolve the powder in distilled water, tube, and autoclave. This medium is called hydrolyzed hemoglobin. It is an excellent medium for the cultivation of intestinal flagellates when the peptone and sugar are omitted. The flagellates grow well and live much longer without subculture than on any medium that we have tried. And it has the advantage of being a wholly autoclaved medium.

10. Prepare the hemoglobin as in medium 9 above and add an equal amount of Difco liver infusion agar to it (removing the agar before use). Then add sufficient glucose to make the mixture 1 per cent. Tube and autoclave. This mixture has also been prepared by the Digestive Ferments Company in a dehydrated form and can be used by dissolving the powder in distilled water, tubing, and autoclaving. This medium is called hydrolyzed hemoglobin and liver infusion or hemoglobin-liver broth.

11. Grind whole potatoes and express the juice through gauze. Then place the liquid in a flask and autoclave. After autoclaving filter through cotton until clear, tube, and autoclave again. Potatoes and carrot may be used as well as apples.

12. Dilute the potato juice prepared as in medium 11 to 15 per cent with 0.8 per cent sodium chloride. Then add sufficient proteose peptone and glucose to make the mixture 1 per cent of each.

13. Cabbage infusion broth as prepared by the Digestive Ferments Company is used. Dissolve 30 gm. of the powder in 1000 c.c. of distilled water, tube, and autoclave.

14. Dissolve the following in 1000 c.c. of distilled water:

10 gm. of Difco dehydrated liver infusion broth
10 gm. glucose
10 gm. proteose peptone
8 gm. sodium chloride
20 gm. of aminoids (prepared by Arlington Chemical Company, Yonkers, N. Y.)

Tube medium and autoclave.

15. Dissolve 30 gm. of c.p. anhydrous glucose in 1000 c.c. of distilled water. Tube and autoclave.

16. Dissolve 10 or 20 gm. of proteose peptone in one liter of either 0.4 or 0.8 per cent sodium chloride. Tube and autoclave.

17. Dissolve 30 gm. of c.p. mannose in 1000 c.c. of distilled water. Sterilize by filtration or by autoclaving.

18. Dissolve 30 gm. of c.p. inulin in 1000 c.c. of distilled water. Sterilize by filtration or by autoclaving. One per cent inulin may also be used.

19. Dissolve 10 gm. of insulin and 10 gm. of proteose peptone in 1000 c.c. of distilled water. Tube and autoclave.

CEREBROSPINAL FLUID: In Premature Infants, Glaser, J. Am. J. Dis. Child. 40: 741, 1930.

From a study of 170 cases Glaser formulates the following conclusions:

Knowledge of the fact that hazy cerebrospinal fluid may be due to acute meningeal congestion permits a tentative diagnosis of this condition when hazy cerebrospinal fluid is obtained by lumbar puncture.

The recognition of this condition increases the diagnostic value of lumbar puncture and, in correlation with necropsy observations, has reduced the percentage of faulty taps to about 14 per cent.

Fifty-five per cent of the yellow cerebrospinal fluids of premature infants give a positive indirect van den Bergh reaction.

A positive direct van den Bergh reaction of the cerebrospinal fluid is suggestive of meningeal hemorrhage.

A positive benzidine test on the supernatant fluid after centrifugating blood spinal fluid obtained by lumbar puncture with proper precautions to avoid hemolysis is proof of meningeal hemorrhage. If the test is negative, however, hemorrhage cannot be ruled out.

The highest icterus index of cerebrospinal fluid observed was 18 and occurred at the age of fifty-two days.

The youngest premature infant from whom a colorless cerebrospinal fluid was obtained was thirty-five days of age.

A case of primary pneumococcus meningitis is reported in a premature infant aged eight days.

A theory is offered to explain the paroxysmal or intermittent cyanosis occurring in premature infants with subtentorial meningeal hemorrhage on the basis of intermittent pressure by blood clot on the medulla oblongata.

A chart is shown, illustrating the relationship between the icterus index of the blood serum and the icterus index of the cerebrospinal fluid. This curve reaches its peak during the second and third weeks of life.

Evidence is advanced in support of a theory that physiologic xanthochromia of the premature newborn infant, and probably of all newborn infants, occurs because of anatomic and physiologic immaturity of the blood-cerebrospinal fluid barrier.

BLOOD: A Simple Oxidase Reaction, Roelofs, B. Nederlandsch. Tijdschr. v. Geneesk. 74: 3524, 1930.

Dopa (Merck), which is O-dihydroxyphenylalanine, is used for staining leucocytes according to Bloch (Ztschr. f. phys. Chem. 98: 226; Ztschr. f. d. ges. exper. Med. 5: 179). Fixed blood smears are immersed for one-half hour in a 0.1 per cent alkalinized solution of dopa; by oxidation a dark brown or black substance (dopa malanin) is formed in the cytoplasma of myelogenous leucocytes, particularly in the granules; also eosinophile and neutrophile cells show the reaction. This reaction is more distinct than the indophenol synthesis from a-naphthol and dimethylphenylenediamine or the oxidase reactions.

URINE: New Test for Bile Salts, Decade, J. Bull. Soc. chim. biol. 12: 130, 1930.

Four drops of alcoholic phthalein solution is added to 10 c.c. of urine in a 30 c.c. tube and 3 times as many c.c. of 0.1 N NaOH added as required for neutralization. An orange-red color develops quite distinct from the violet-red obtained with normal urine.

DYSENTERY: New Method of Identification of Type, Ghosh, H. Compt. rend. Soc. de Biol. 103: 55, 1930.

Use feces obtained as soon as possible after onset of illness. To 20 c.c. of feces add 0.5 per cent $CHCl_3$; mix well; and incubate for thirty minutes. Filter through fine muslin, then through Pasteur-Chamberland boughie L2 under light pressure. To get a filtrate quickly a little physiologic saline solution may be added. Take 4 c.c. of the filtrate; in each of two tubes place 1 c.c. of the filtrate and 0.2 c.c. of anti-Shiga serum. Add to

control tube one drop of 1 in 5 commercial formaldehyde. Incubate both tubes in water-bath for one hour. A positive reaction is shown by appearance of fine flocculi, distinctly seen by lens. The control is quite clear. If serum contain precipitate it must be cleared by centrifugation.

TRYPANOSOMIASIS: Flocculation Test for, Sica, A. Bull. Soc. path. exot. 22: 912, 1929.

One hundred gm. of minced heart muscle is allowed to macerate in 125 c.c. of 95 per cent alcohol for seven days at 20° C. Filter and drain the residue; this is then spread on a glass plate and allowed to dry for twenty-four hours at 37° C., after which it is placed in 200 c.c. of acetone and allowed to stand for eight days at a temperature of over 20° C. Filter, drain, and rinse the fragments and place them in a little more acetone for twenty-four hours in order to ensure the removal of all acetone soluble material. Filter, drain, and dry the residue at 37° C. for four hours. The dried residue is then weighed and allowed to macerate in a closed flask with absolute alcohol, 100 c.c. of alcohol for each 15 gm. of dried residue. The maceration is continued for twelve days at over 20° C., the flask being shaken each day. Filter: the fluid which passes through represents the alcoholic extract of the acetone residue. It should be preserved in a sealed bottle.

As diluting fluid the author has tried various concentrations of sodium chloride in distilled water and finally has decided on 3.5 per cent, which gives undoubtedly the best results. The serum of the patient must be perfectly clear, containing neither hemoglobin nor bile pigments, and must be freshly obtained, and not heated.

The test is conducted as follows: Into one tube is dropped from a pipette 1 c.c. of the alcoholic extract of acetone residue of the heart, and into another 14 c.c. of the 3.5 per cent NaCl solution. The two tubes are left in the water-bath at 40° C. for ten minutes, and then the contents of the second tube are poured into the first and that of the first into the second and so on until the two solutions are completely mixed and a perfectly homogeneous, very slightly opalescent fluid is obtained. This is left in the water-bath at 40° C. for ten minutes and then 1 c.c. of it is added to each of the tubes containing 0.1 c.c. of the various sera to be examined. The mixture is shaken and then allowed to stand.

With the object of hastening the reaction, the author investigated the various essences at his disposal and found that essence of cloves diluted with 50 times its volume of absolute alcohol when added to the heart extract greatly increased the rapidity of the reaction. With this modification 1 c.c. of alcoholic extract of muscle is added to 1 c.c. of the 1/50 solution of essence of cloves in the first tube and the 15 c.c. of the 3.5 per cent sodium chloride in the second. The mixture is then made as before.

The sera of uninfected persons gives no reaction, the contents of the tubes remaining perfectly clear. But the sera of infected persons produces within a few moments a definite turbidity; later flakes appear, varying in size and number and gradually fall to the bottom of the tubes. The reaction is complete within twelve hours.

TRYPANOSOMIASIS: Targowla's Reaction, Le Gac, P. Bull. Soc. path. exot. 23: 530, 1930.

The reagents required are: (1) recently distilled water; and (2) pure exilir of paregoric prepared according to the Codex. The apparatus necessary consists of two hemolysis tubes and two dropping pipettes of the same caliber, i.e., delivering 50 drops of paregoric per c.c. The glassware must be thoroughly cleaned with 2 per cent HCl, and then carefully rinsed with distilled water and dried.

The test is performed as follows: Into one hemolysis tube add successively 5 drops of distilled water, 15 drops of cerebrospinal fluid, and 5 drops of elixir of paregoric; shake well to obtain a homogeneous mixture.

Into the second hemolysis tube (control tube) are placed 5 drops of distilled water and 5 drops of elixir of paregoric. The tubes are left at laboratory temperature and the result read after twelve to twenty-four hours.

+++ indicates total precipitation.
++ indicates partial precipitation.
+ indicates slight precipitation.
– indicates no precipitation (negative reaction).

The test was examined in 9 cases of sleeping sickness—4 at the commencement of the second stage, 3 definitely in the second stage, and 2 in the third stage.

Of the 4 cases in the first category, 2 gave a +++ reaction, 1 a + reaction, and 1 a negative reaction. All the cases in the second and third categories gave a +++ reaction.

CEREBROSPINAL FLUID: Estimation of Albumin in, Armand, R. Bull. Soc. path. exot. 23: 383, 1930.

The author presents a modification of his previous method which proved unsatisfactory.

The cerebrospinal fluid (4.3 c.c.) is put in a Siccard's tube, heated to boiling, and then 0.5 c.c. of the following reagent is added:

Trichloracetic acid	10.0 gm.
Alcohol, 95 per cent	30.0 c.c.
Carbon tetrachloride	2.5 c.c.

The mixture is then well shaken and allowed to stand.

The author claims that with this solution it is possible to read the results from twenty to thirty minutes later; whereas with the classical procedure five hours are required.

FOCAL INFECTION: Production of, Moon, V. H., and Kanzelmann, F. W. Arch. Path. 10: 587, 1930.

Applicators of cotton, similar to those commonly used for sore throat swabs, are made on No. 20 or 22 rustless wire rather than on wood. The cotton is wound rather loosely, so that moderate force will slip it off the wire. A trocar and cannula are inserted into the region to be inoculated. The trocar is then withdrawn, leaving the cannula in place. The cotton applicator is dipped into the material to be inoculated and is inserted through the cannula. The swab is pushed beyond the end of the cannula, then, on withdrawing the wire, the end of the cannula pushes off the cotton into the tissue or cavity desired to be infected. The cannula is then withdrawn.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

A *Textbook of Histology*[*]

THIS volume, completed after the death of Professor Maximow by his associate Dr. William Bloom, is a distinct contribution to the subject.

The original plan, of a textbook of histology based mainly on human material and with special emphasis upon the functional aspects of the structures described, has been closely followed. The result is a text which should prove of value to the histologist, the pathologist, and the physician as well as to the student.

It will without doubt become a standard reference text.

The typography, illustrations, and general craftsmanship are excellent.

Both the author and the publishers are to be congratulated.

Intestinal Toxemia[†]

THAT within the thirty-two feet of the intestinal canal, with its varied and varying bacterial population, a multiplicity of chemical and biological reactions and interreactions are constantly occurring with the formation of varied and often little understood products, has long been matter of common knowledge.

To what degree these substances, the nature of many of which is still problematical, may be absorbed, and to what extent they may influence the organism as a whole or, in other words, their exact relation to, and importance as causes of disease, are matters of dispute, debate, and contention.

The subject in all its ramifications is one in which Dr. Bassler has long been interested and to the elucidation of which he has contributed years of clinical study and laboratory investigation and the present volume presents in full detail his position in the matter based upon a study of 5000 cases and extending over thirty years.

As such it deserves the perusal of the profession.

It is, indeed, refreshing, in these days of "colon-filling stations," at present passing through the recrudescence of popularity, to see this subject reviewed in an intelligent and sane manner, and to read of the rise and fall of other fads and fancies in a field fertile in their production.

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[*]A Textbook of Histology. By Alexander A. Maximow, Late Professor of Anatomy, University of Chicago. Completed and Edited by William Bloom, Assistant Professor of Anatomy, University of Chicago. 833 pages with 604 illustrations, some in colors, Philadelphia and London, W. B. Saunders Co., 1930.

[†]Intestinal Toxemia (Autointoxication). By Anthony Bassler, M.D., F.A.C.P. Cloth, 433 pages, 15 illustrations, four charts, F. A. Davis Co., Philadelphia.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

A large portion of the volume is given to a discussion of intestinal bacteriology, a subject certainly not clearly outlined as yet, and to the methods adapted for the study of the intestinal contents and the clinical interpretation of the results.

The urine and blood, as well as the examination of the patients are all covered in detail. The various methods of treatment, dietetic, bacterial, biologic and so on are all passed in review with pungent comments upon their value and their underlying principles.

The relation of intestinal toxemia to the various fields of medicine is considered at length and in detail.

Included in the work are four very comprehensive and useful charts concerning the characteristics of the intestinal bacteria.

The volume represents a long, comprehensive, and careful study and constitutes a distinct contribution to a very confused subject of distinct clinical importance.

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*Intestinal Tuberculosis**

THE fact that intestinal tuberculosis was not, as had formerly been thought, mainly a terminal complication of pulmonary tuberculosis was first emphasized by the authors in the first edition of this work.

Since that time their work, and the conclusions based upon it, have been largely substantiated by the investigations of others.

In the present edition, which has been amplified and brought up-to-date, the methods utilized by the authors in the diagnosis of intestinal tuberculosis are described at length and in such detail that they may be carried out by those who desire to use them.

The method of roentgenological diagnosis, first perfected and interpreted by the authors, is explained in full detail, with numerous text illustrations.

The volume is a noteworthy contribution and may be recommended without reserve as an authoritative survey of a very important subject.

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*Intestinal Tuberculosis, Its Importance, Diagnosis, and Treatment. A Study of the Secondary Ulcerative Type. By Lawrason Brown, Consultant, and H. L. Sampson, Roentgenographer, to the Trudeau Sanitarium. Second Edition, Cloth, 376 pages, 122 engravings, two colored plates. Lea and Febiger, Philadelphia.

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EDITORIALS

Direct Culture, or Guinea Pig Inoculation, for the Discovery of Tubercle Bacilli

ISOLATION of the tubercle bacillus from sputum or other contaminated material was for a long time one of the most difficult problems of bacteriology. The original method of Koch and Kitasato consisted in thoroughly washing a small piece of sputum in several dishes of sterile water before planting it on coagulated serum. The results were not very satisfactory; it was largely a matter of luck if the culture would be contaminated or not, and growth was always slow on the serum medium.

At present there are more than a dozen methods to obtain a pure culture of tubercle bacilli from sputum. All of them give good results. The introduction of antiformin by Uhlenhuth was the first improvement over the old method; the antiformin destroyed the contaminating organisms, but left the tubercle bacilli intact. Other chemicals now in use for the same purpose are sodium hydroxide (Petroff), sulphuric acid (Loewenstein, Hohn), oxalic acid (Corper), and several others of less importance. Petroff's method, using

sodium hydroxide is widely used in this country, but Loewenstein's sulphuric acid method as improved by Hohn is employed by many European workers, and has lately been advocated by Corper. In this method 6 per cent sulphuric acid is mixed with the sputum; the mixture is shaken for half an hour, centrifuged and the sediment planted on the medium. The advantage of this method over those using antiformin or sodium hydroxide is that the sediment does not need to be washed or neutralized; this saves time and lessens the chance of contamination.

Another improvement over Koch's method is the use of new media. Their principal ingredient is usually egg (Petroff, Hohn) or potato (Loewenstein, Corper); some authors find benefit in the addition of milk (Petragnani), cream (Sweeny), or hematin (Hohn). Bacteriostatic dyes as gentian violet (Petroff), crystal violet (Corper), or malachite green (Petragnani) may be added to prevent the growth of saprophytes, but are omitted by many authors. The choice of one of these media seems largely a matter of personal preference or experience, and excellent results are obtained with all of them.

The results of culturing the tubercle bacilli from suspected material are claimed to be as accurate as the animal experiment. Outstanding advantages of the culture method are cheapness, need for less laboratory space and the fact that less time is necessary for arriving at a positive diagnosis. The first factor has greatest weight in many European countries and the second will be of importance in most hospitals. With the culture method it is possible to make daily cultures in doubtful cases and thus to increase the chance of finding the bacilli; this would never be feasible with animal experiments. Positive evidence of growth may be obtained as early as the fifth day and usually within twenty days, while the guinea pig experiment takes six weeks or longer.

The principal advantage of the guinea pig method is that it calls for less technical skill and may be performed by any physician without laboratory training. In addition, no incubator, centrifuge or inspissator are needed.

The animal experiment provides at the same time a virulence test of the bacilli. There are however two sides to this question. Hohn praises the culture test because it permits the discovery of strains of tubercle bacilli pathogenic for man but not for guinea pigs. It must be very difficult to prove the existence of such strains, except by human experiment. Dimtza reported the isolation of such a strain by the culture method from a case of clinically benign kidney tuberculosis. Most workers would consider this as a smegma bacillus, and it is difficult to see how the isolation of a nonpathogenic acid-fast bacillus from the urine can help the physician in arriving at a diagnosis.

In autopsy material it will be necessary in future to consider the possibility of the presence of the B.C.G. bacillus; in this case a combination of culture and animal virulence test will be needed.

A recent study by Corper* has contributed much to our knowledge of the culture of the acid-fast saprophytes. He found that sulphuric acid, oxalic acid and acetic acid do not inhibit the growth of the smegma bacillus. Hydrochloric acid and sodium hydroxide on the contrary prevent the growth of

*Corper and Uyei: Oxalic acid as a reagent for isolating tubercle bacilli and a study of the growth of acid-fast non pathogens on different mediums with their reaction to chemical reagents, J. Lab. & Clin. Med. 15: 348, 1930.

all acid-fast bacilli, except the tubercle bacillus. These last two reagents are accordingly to be preferred for killing the contaminating bacteria in urine.

Whether the culture method or the animal test is preferred will depend largely on the laboratory facilities that are available. For an occasional test the guinea pig experiment is certainly much simpler, but in large institutions where many tests are performed by skilled workers the culture methods present important advantages.

—*G. B. W.* (*C. H. B.*)

Errata

In the article by Youngburg and Youngburg, November, 1930, page 158, "Phosphorus Metabolism. I. A System of Blood Phosphorus Analysis," the following errors in calculation appeared:

On page 164 calculation for serum acid-soluble phosphorus and calculation for serum total acid-soluble phosphorus should read 20/reading × 5 = mg. P per 100 c.c. blood plasma or serum.

On page 165 calculation for plasma or serum total phosphorus should read 20/reading × 20 = mg. P.

On page 165 calculation for total acid-soluble phosphorus in corpuscles should read 20/reading × 50= mg. P.

On page 165 calculation for total phosphorus in corpuscles should read 20/reading × 80 = mg. P.

On page 165 calculation for total phosphorus in whole blood should read 20/reading × 40 = mg. P.